https://dx.doi.org/10.21577/0103-5053.20230096

J. Braz. Chem. Soc., Vol. 34, No. 11, 1542-1564, 2023

Review

Capillary Electromigration Techniques Applied to Clinical Chemistry Investigations and Alternative Diagnostic Assays: Possibilities and Perspectives

Olívia B. O. Moreira,[a] Bruna M. Berlande,[a] Jéssica C. Q. de Souza,[a] João Marcos B. Candido,[a] Lauren H. Jaeger,[b] Luiz Henrique C. Adriano,[a] Marco Antônio D. A. e Brito,[a] Maria Patrícia do Nascimento,[a] Patrícia A. Geraldo,[a] Paula R. Chellini[a,b] and Marcone Augusto L. de Oliveira[*,a,c]

[a]*Grupo de Química Analítica e Quimiometria (GQAQ), Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Juiz de Fora (UFJF), 36036-900 Juiz de Fora-MG, Brazil*

[b]*Faculdade de Farmácia, Universidade Federal de Juiz de Fora (UFJF), 36036-900 Juiz de Fora-MG, Brazil*

[c]*Instituto Nacional de Ciência e Tecnologia de (INCTBio), Instituto de Química, Universidade de Campinas (UNICAMP), 13083-970 Campinas-SP, Brazil*

Capillary electromigration techniques can be understood as the ones used to separate neutral compounds, solvated ions and ionized species, considering the differentiated migration among them when an electrical field is applied within a capillary column filled with a background electrolyte. Several existing capillary electrophoresis setups provide the possibility of developing reliable quantitative assays of inorganic ions, organic acids, fatty acids, amino acids, carbohydrates, nucleic acids, proteins, hormones, vitamins, macromolecules, and more. Therefore, given the chemical complexity of bodily fluid matrices, capillary electrophoresis (CE) and its variations present themselves as an advantageous strategy for enhancing traditional clinical diagnostic methods and for the development of new ones. The recent advances in the study of the human metabolome associated with technological improvements towards medical applications make CE a very useful and versatile technique for clinical laboratory assays. Within this context, this review has the purpose of presenting some traditional methods used for exams of blood, urine, saliva, feces, and sweat, and how CE can be implemented as a real alternative to enable faster, automated, and cost-effective analysis with a comprehensive perspective that shall result in better diagnostic possibilities for patients, being a powerful tool for helping physicians on the precision medicine achievements.

Keywords: capillary electrophoresis, precision medicine, diagnosis, clinical analysis

1. Introduction

Capillary electromigration techniques were introduced as a new separation alternative in the early 80s and since then its potentialities have been discussed and exploited by scientists from different areas of expertise, especially the ones that consider bioanalysis.[1,2] Back in the beginning of the 90s decade, the application of analytical methods based on electromigration was already being considered for clinical diagnostic purposes. As highlighted by Chen *et al.*,[3] the new advances brought at the time to traditional electrophoresis, would enhance the proteins and deoxyribonucleic acid (DNA) analysis by offering automation, less time-consuming and labor-intensive routines. As the authors anticipated, the analysis of DNA fragments, ribonucleic acids (RNA), proteins, and such are performed today by automated capillary gel electrophoresis systems.

Over the last decades, a series of reviews[4-12] were published to discuss other possibilities for the implementation of capillary electromigration/electrokinetic-based techniques in clinical laboratories to enhance several ongoing conventional methods. Overall, electrophoretic separation in capillary columns happens over the differentiated electromigration characteristics of

*e-mail: marcone.oliveira@ufjf.br
Editor handled this article: Andréa R. Chaves (Associate)

the analytes when submitted to an electrical field within a capillary fill up with a mediator background solution.

The promising features of these techniques come from their high-efficiency, micro/nano-scale consumption, versatility, selectivity and more. They present some inherent advantages regarding method optimization procedures, being possible to consider as variables pH, viscosity, background electrolyte (BGE) concentration, voltage, temperature, injection mode, detection, and associated parameters. Altogether, capillary electrophoresis (CE) usually allows short-time runs, even with sub-minute possibilities, without undermining resolution and selectivity that enhance analytical frequency, which is already a competitive advantage compared to other separation techniques itself. Besides, it is a cost-effective protocol, given the reduced consumption of solvents and reactants, including hazardous substances, which consequently implies on Green Chemistry precepts.

Capillary zone electrophoresis (CZE) is the simplest and most easily CE mode implemented, thereby being an excellent strategy for several purposes.[1,2,13] However, given the intrinsic proprieties of the compound under investigation, it all can be adapted towards to what the analysis needs, hence the versatility and selectivity features of the technique, where all can be aligned towards the registration of a specific substance or group of substance. In this context, there are several CE modes (Figure 1) that can be diversly applied, either isolated or combined, by using the same equipment with all advantages aforementioned.

By using the CE modes described in Figure 1, it is possible to selectively analyze solvated ions, ionizable species, and sorts of other compounds like neutral molecules, amphoteric compounds, hydrophilic and hydrophobic species through micellar electrokinetic chromatography (MECK), microemulsion electrokinetic chromatography (MEEKC), capillary electrochromatography (CEC), capillary zone electrophoresis (CZE) and even non-aqueous capillary electrophoresis (NACE), a variation of CZE.[14] All that with the advantage of not needing molecular derivatizations and other sample pre-treatment procedures. Therefore, CE provides us the ability to analyze several groups of molecules in any bodily fluid with less laborious and less time-consuming processing is an excellent fit for clinical laboratories. As previously commented, capillary gel electrophoresis (CGE) has been very useful for macromolecules (proteins, enzymes) analysis and as an auxiliary tool for DNA sequencing, as capillary isoelectric focusing (CEIF) can be also useful in this regard. Capillary isotachophoresis (CITP) can be used for sample preconcentration, separation, purification, mixing and to control and accelerate chemical reactions.

Additionally, CE can be coupled to several detectors that contribute to selectivity and improve sensitivity, such as diode array detection (DAD), mass spectrometry (MS), laser induced fluorescence (LIF), capacitively coupled contactless conductivity detector (C^4D), which all can be adapted as needed. Finally, there are also possibilities involving microchip and similar microdevices systems useful for several applications.

Given the chemical complexity of the biological fluids from the human body, the possibility of implementing capillary electrophoresis in clinical diagnostic laboratories is presented with great potential. Other than that, new technologies have already been applied and are still being developed towards the automation and quality enhancement of quantitative diagnostic assays,[15] aiming at better results

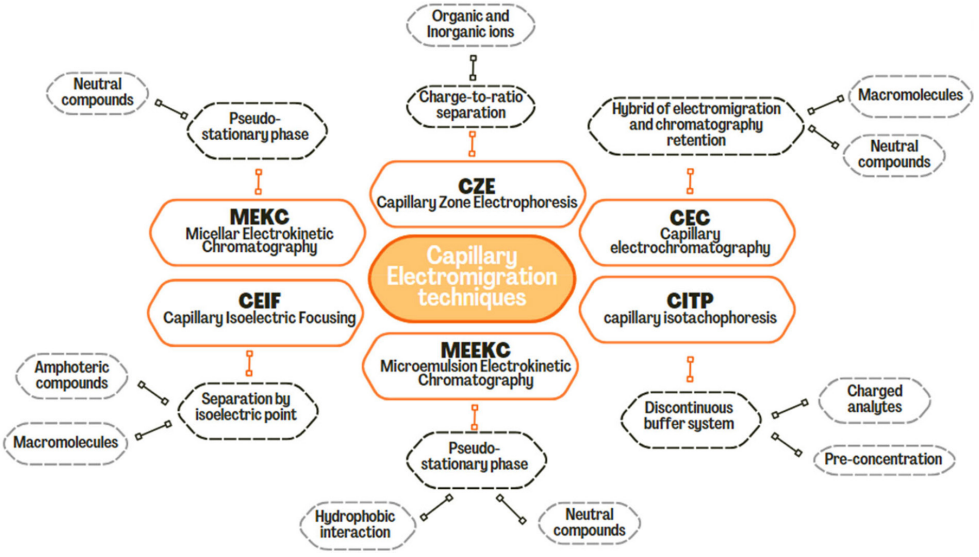


Figure 1. Summary of the main capillary electromigration modes and their respective applicability.[1,2,12,13]

with faster delivery and, more important, new assessments of health-related issues.

In a recent review published by our research group,[16] we discussed the potential of capillary electrophoresis as an option of instrumentation for urine diagnostic tests. Following this line of thinking, our approach in this work was to do a study of cases where we discuss electromigration methods as a real advantageous alternative for specific tests and why diagnostic clinical laboratories should implement a CE equipment in their facilities. We will be presenting new alternatives based on CE methods not only for analysis of urine, but also for exams based on analysis of blood parts, feces, sweat and saliva, commenting on the existing methods and ways of improvement.

2. Urine

Urine is the primary resource of mammals to eliminate waste products. It is a sterile, easy to collect fluid that results from the kidney activity. As a solution originated by the water-soluble residual chemicals of our bloodstream, it contains inorganic salts, creatinine, urea, ammonia, organic acids, toxins, pigments, and countless other substances.[17] Therefore, it provides a lot of information about the human health and so it is the oldest clinical laboratory test ever developed.[18,19]

Urinalysis is a comprehensive and important diagnostic screening test that includes complete physical, chemical, and microscopic examination prescribed by physicians for both adult and pediatric patients.[18-20] According to Kouri *et al.*,[21] the test should be performed based on the medical need with appropriate examination and be presented to the patient considering the cost/benefit aspects. A complete urinalysis is usually suggested to investigating kidney malfunctions and diseases associated to the urinary tract.[21]

Overall, urinalysis considers pH, color, odor, specific gravity (USG), glucose, ketones, bilirubin, occult blood, proteins together with urinary sediment evaluation, which the object of investigation are erythrocytes, leukocytes, bacteria, crystals, and granules.[20,22-24] Regardless, given the chemical complexity of human urine, it is considered an outstanding bodily fluid for the study of the metabolic products.[17] Therefore, a comprehensive amount of other diagnostic tests are offered by specialized laboratories to verify several anomalies other than the ones related to kidney and urinary tract. All the aforementioned tests are performed through visual examination, dipstick tests, colorimetric reactions, liquid chromatography, mass spectrometry, and more. For some specific ones, new technologies based on CE could be an enhancement, as we will be discussing in this section.

2.1. Salt measurements

Some inorganic salts are routinely tested in urine. For sodium and chloride, a few rapid tests are commercially available mostly used for monitoring NaCl intake.[25] Together with potassium and nitrate, they are measured by ion-selective electrode (ISE) and colorimetric reactions.[26-28] The measurement of these inorganic salts gives the patients a perspective of their electrolyte balance. Such imbalances are directly associated to hydration, hypertension, hormones secretion anomalies, inappropriate diuresis, muscle and nerve damage, eating disorders, acidosis, fibrosis, infections and more.[26,29-32]

Stripe tests like dipstick urine tests or rapid tests usually give a qualitative response or a range of concentration of some inorganic salts. It is found in the specific literature some reviews on the accuracy of these tests.[25,33-35] According to Roberts,[36] emergency physicians order these analyses routinely when they can fully interpret the results. James *et al.*[35] comment that patients themselves can easily perform the test, which we believe it can be very useful for an instant assessment of a chemical panel. Regarding the ISE and colorimetric possibilities, since ISE uses unique chemical properties to measure these analytes, it provides good selectivity and sensibility[26] in the absence of interferents, what it may not be true for urine.[27,28] Meanwhile, the colorimetric method can also be influenced by other chemicals, in addition to taking more time and it usually requires the use of aggressive chemicals, such as mercury thiocyanide for chloride determination, for example.[27]

In cases where a more accurate quantitative analysis is needed to evaluated non-emergency conditions, diagnostic tests using CE for inorganic salts could be developed. As commented in a previous work,[16] ions are part of the basic fundamentals of CE separation mechanism, and so it is a strategic fit for exams as such. Overall, analyses of anions require the use of electroosmotic flow (EOF) inverter plus some chromophore agents to allow the UV detection. For this reason, it is common to find studies in which electrochemical-related detectors as C^4D are prioritized, with or without microchip capillary electrophoresis (MCE) possibilities.[37-42] This approach is usually faster than UV-detection alternatives and more sensible. Interesting evaluations are found in these studies, as the interfering features of background electrolyte if compounds that may form a complex with the ions is present[37] and the influence of hydrodynamic *versus* electrokinetic injection on resolution.[43] The method that stands-out considering the

analysis of ion is the one developed by Yamamoto *et al.*[44] that can simultaneously detect both anions and cations in the same run with total time of approximately 15 min. In this context, an interesting review by Sáiz *et al.*[45] include some good discussions on challenges and theoretical aspects of this kind of simultaneous analysis.

On the other hand, ultraviolet (UV) detectors are intrinsic to the equipment and so could be easier to implement, and can provide comparable outcomes (Figure 2). Additionally, the analysis of ions may require ionic strength adjustments, since it could be adjusted within the BGE, the samples do not necessarily require extensive preparations. Regardless, a first attempted to input these methods in laboratory routines can be based on the adaptation of simpler apparatus, as CE with DAD lamp with separated methods as the ones exemplified at Table 1 in the Overview section. Overall, UV-based methods also provide quantitative analysis in micro-molar scale within minutes, which is enough to dose these analytes in urine. Figure 2 shows an example of CE optimized methods applied on the analysis of inorganic cations and anions separately, both in waste water samples, that can be adapted to analysis of urine or other bodily fluid that may seem relevant.

Other than that, a new device developed by Wang *et al.*[48] offers simultaneous detection by C^4D, LIF, and UV by using a 3D-printing detector. The device was tested in the aforementioned inorganic salts and other compounds. This kind of new technologies coupled to CE, or similar ones based on the electromigration phenomenom, are exactly what we believe will be the new competitive advantage of CE in the near future. Devices that can miniaturize and enable large-scale batches of analysis at a lower cost can be used as dedicated equipment for every needed analysis in the future, thereby being possible to offer more results in less time, with reduced systematic errors and amplified automatization.

2.2. Main urine components

Still considering colorimetric analysis, some organic species are also dosed by colorimetric methods. Among them, creatinine is a marker of renal function and a normalization for other molecules measured in urine that usually are expressed as a ratio to creatinine. Creatinine is also measured in serum samples to estimate glomerular filtration rate (eGFR) that punctuates the filtration capacity of the kidneys.[49,50] Traditionally, creatinine is estimated through the colorimetric reaction with alkaline picrate with spectrophotometric detection.[51] Aside from the common interferents for urinalysis (bilirubin, hemoglobin, and triglycerides, at a certain concentration), as highlighted by Saibaba *et al.*,[52] other substances can interfere directly to creatinine determination like ascorbic acid and some drugs.

Regarding the other major components of urine, urea is determined indirectly as an indication of protein metabolism by coupling-reaction with urease and glutamate dehydrogenase (GLDH) as enzymatic mediators. The sodium hypobromite test and urease test, that considers phenol indicator to the urea reaction to Na_2CO_3, are also presented as a qualitative protocol.[53,54] Also an indicator of protein metabolism, uric acid is analyzed by a coupling-reaction based on the enzymatic assay with urease and peroxidase as catalyzers to produce the detectable colored product.[55]

Enzymatic assays are usually specific, however, from an analytical perspective, the direct reaction by a separation technique will offer less laborious routines in additional to reducing the error propagation and interferences.

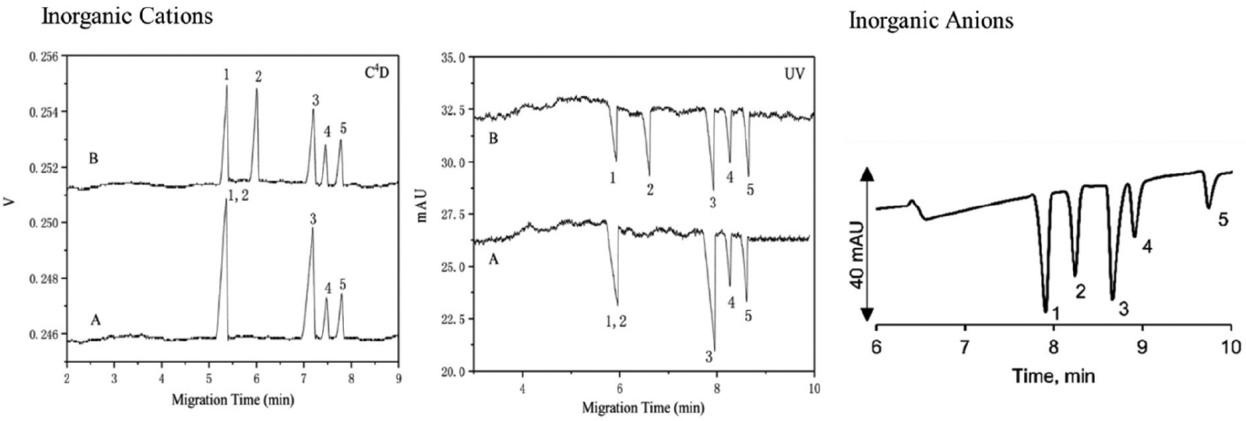


Figure 2. Electropherograms resulting from the analysis of inorganic cations by CZE under C^4D and indirect-UV detection, and inorganic anions by CZE with indirect-UV detection. Inorganic cations: method A (without 18-crown-6) and method B (optimized, with 18-crown-6). (1) NH_4^+; (2) K^+; (3) Ca^{2+}; (4) Na^+; (5) Mg^{2+}. Optimize electrophoretic conditions described in Table 1 (reproduced from Zhao *et al.*[46] with copyright permission 2021 from Royal Society of Chemistry). Inorganic anions: (1) Cl^-; (2) NO_2^- (3) NO_3^-; (4) SO_4^{2-}; (5) HCO_3^-.Optimize electrophoretic conditions described at Table 1 (reproduced from do Nascimento *et al.*[47] with copyright permission 2022 from Royal Society of Chemistry).

Overall, the analysis of urine by CE usually requires only a deproteinization procedure and dilution, if so. Another advantage is the possibility of using internal standards that enhance the method reliability and quantitative precision.

A considerable number of CE-methods for creatinine determination is found in the literature since the 90s, just with a dynamic search on the literature countless classical studies are found. Creatinine (pK_a 4.8)[56] is a chromophore-containing ionizable compound that can be easily analyzed by capillary zone electrophoresis with UV detection (CZE-UV). Over the last five years, some straightforward evaluations and devices were elaborated.[57] Piestansky *et al.*[58] compared ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) and CZE-UV already considering a clinical perspective and concluded that UHPLC-MS/MS may be the outstanding option given the better sensitivity, specificity, and accuracy. On the other hand, the authors argue that CZE-UV methods are reliable yet simpler, with "diluted-and-shot approach", and with sensitive range within needed, which we agree. Recently, a study developed by our group[56] is based on the determination of creatinine in urine and blood serum by CZE-UV with internal standard on-column injection that results in a 5 min method. In that work,[56] we compare the urinary creatinine quantification got from the optimized proposed method against the gold-standard by titration with alkaline picrate, as a result, we have seen the influence of the aforementioned interferents and the extensive analytical process on the sub quantification of the colorimetric protocol.

Not so much is true for urea and uric acid; some authors[59] consider the analysis of uric acid (pK_a 5.4) by CE with electrochemical detection. In a recent review,[60] two methods based on CZE with electrochemical and chemiluminescence were discussed. Some UV-methods applied in saliva dates from the early 2000s and it is compared with other possibilities by Vernerová *et al.*[61] Uric acid is also determined in blood as routine exam for both gout and other anomalies, thus, more in uric acid will be discussed in the next section. A good but promising challenge would be somehow developing a method for simultaneous determination of the three of them, perhaps considering MEKC for urea (pK_a 0.1)[62] plus CZE combined, which would be an interesting alternative as a direct quantification assay.

2.3. Comprehensive approaches

An interesting work was developed by Sidorova and Grigoriev[39] in which the authors consider the CE analysis of all markers for urolithiasis (popularly, kidney stones). Four CZE-UV methods were developed and validated for (*i*) inorganic cations; (*ii*) inorganic anions; (*iii*) uric acid and creatinine; (*iv*) citric and oxalic acid, thereby being a good start for implementation as laboratory exam.

Due to advances in omics sciences and mass spectrometry, the clinical laboratories have been offering to patients health/nutritional full panels considering key groups of metabolites. Amino acids, organic acids, vitamins, short-chain fatty acids (SCFAs), and minerals are a stand-out.[63-66] Inductively coupled plasma mass spectrometry (ICP-MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS) are the techniques in which the exams are performed.

From experience, when urinary analysis is the focus, the number of metabolites is massive, thereby being a challenge to propose quantitative analysis of groups of molecules in a single run without use unequivocal detection, like mass spectrometry. In some cases, besides the ones aforementioned, the isolated analysis of a few analytes with UV detection can be easily achieved with great selectivity and should be considered in the routine of clinical laboratories. However, chemical groups like amino acids or organic acids might require an association with mass spectrometry, on the other hand, this coupling can also be with a capillary electrophoresis equipment. The advantage here comes from the eco-friendly features of CE combined with the possibility of faster runs and lower cost. We do not believe that LC-MS/MS would be replaced, but having a capillary electrophoresis-mass spectrometry system (CE-MS) as an auxiliary equipment to follow high-demanding laboratories may be a competitive advantage.

For example, amino acid screening is also a currently-offered laboratory exam, performed by LC-MS/MS,[64] where CE-MS coupling might be an alternative. Piestansky *et al.*[67] successfully quantified the 20 proteinogenic amino acids in urine with concentration average of 3-500 μmoL L^{-1} using a tandem mass spectrometry system coupled to CE (CE-MS/MS). Those levels are consistent with the value found for real samples submitted to the commercially protocol.[64]

Same comments go for toxicological screenings. Usually, urine is the bodily fluid of preference for detecting metabolites of illicit drugs like cocaine, amphetamines, opioids, benzodiazepines and more. In this context, a study published by DiBattista *et al.*[68] is based on multisegmented injection-capillary electrophoresis-mass spectrometry (MSI-CE-MS) for screening drugs of abuse, where the authors were able to successfully analyze opioids, stimulants, and benzodiazepines in urine with serial injections of 10 samples. Given the chemical diversity of these compounds (Figure 3), by using the

versatility of electrophoresis regarding the different separation mechanisms that can be applied, CE-MS or other associations can be considered as well. In some cases, like opioids, if the separation of isomers is relevant, some good resolution may be achieved by using cyclodextrins, for example.

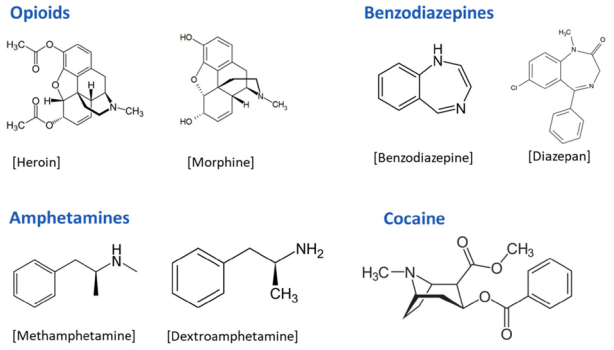


Figure 3. Chemical structures of examples of common drugs set as biomarkers in toxicological screens.

Some CE-based methods focused on the analytes discussed in this section are summarized in Table 1 in Overview section. We prioritized examples of simple but high-throughput alternatives and new ideas published over the last few years. In some cases, the studies were done in different samples other than urine but it can be adapted, since the analysis of urine usually takes only a deproteination step. Regardless, a thorough evaluation of methods and possibilities for urine, specifically, is described in our previous work.[16]

3. Blood

Blood is a body fluid composed of red and white blood cells, platelets, and a liquid carrier named plasma that corresponds 50-55% of total blood volume.[17] The plasma itself can be separated from the blood through the use of anticoagulants and simple centrifugation. If the blood clots without the use of anticoagulants, the supernatant fluid is named serum, which plays an essential role in transporting dissolved gases, regulating pH and ionic composition of interstitial fluids, acting in the defense mechanism against toxins and pathogens, stabilizing body temperature and more.[17,69]

As blood circulates through every tissue and every organ in the body, it essentially functions as a liquid highway for all the molecules secreted by different tissues in response to different physiological conditions.[17] In this way, it contains a variety of substances such as amino acids, vitamins, hormones, lipoproteins, enzymes, lipids, carbohydrates, electrolytes, and a variety of other small organic molecules suspended or dissolved in them. As a result, most clinical tests are based on the analysis of blood plasma or serum since tissue lesions, organ dysfunctions and pathological states can alter the chemical and protein its composition.[17,69]

3.1. Amino acids

The quantification of some amino acids in blood and urine are tests indicated for the suspicion of several aminoacidopathies and other metabolic diseases.[70] Within this context, high levels of homocysteine (Hcy) in plasma or serum may be associated with the prevalence of cardiovascular diseases,[71,72] atherosclerotic vascular disease of the coronary, cerebral, and peripheral vessels, in addition to thromboembolism.[73]

The measurement of Hcy levels in plasma are based on high performance liquid chromatography (HPLC) as a reference technique.[74] However, clinical laboratories employ chemiluminescence, spectrophotometry, enzymatic cycling, and immunoassays.[75] Several studies[74-77] highlight that the analytical performance of these techniques can be compared satisfactorily with the one considered as a reference. However, they have some limitations, as commented in the previous section. CE can also be an analytical technique used in the quantification of amino acids.[78] Ivanov *et al.*[79] described a method capable of simultaneously quantifying cysteine (Cys) and Hcy in human blood plasma and urine, using CZE-UV within 4 min, demonstrating that the Cys/Hcy ratio in patients with kidney disorders was significantly lower than the one obtained in the control group, as expected. Although the method requires a 90-min derivatization step, this procedure reduced the influence of matrix components on the injection and analysis. Cieslarova *et al.*[80] described two methods for determination of Hcy, cysteine and methionine, and glutamic acid in human plasma by capillary electrophoresis tandem mass spectrometry (CE-MS/MS) within 25 min. Another study by Miyaki *et al.*[81] described a method for simultaneous analysis of Hcy and glutathione in human serum by microchip electrophoresis with laser-induced fluorescence detection. Different concepts of detection may be needed, since considerable low limit of detection is required. In a clinical diagnostic laboratory, using the chemiluminescence technique, the plasma homocysteine test has as reference values of 5.46 to 16.20 μmol L^{-1} for men and 4.44 to 13.56 μmol L^{-1} for women. Therefore, the use of MS systems or other devices that enable sensitive and selective runs might be required. Altogether, these and other amino acids can be analyzed in blood, if relevant, by adapting the protocol mentioned in the previous section.

Given the preanalytical factors for distinguishing normal levels of Hcy in plasma from those individuals at high risk of neurodegenerative and cardiac diseases, some parameters may influence the amount of this amino acid in the blood, associating its dependence with gender, age, renal functions, genetics, drugs, and nutritional status which is influenced by the absorbed amount of folic acid, vitamins B_6 and B_{12}, and total dietary protein content.[82,83] In addition, another source of variation can come from erythrocytes, since they still produce and release Hcy into plasma.[84]

3.2. Vitamins

Vitamins are organic compounds that are essential to health because they can participate in enzymatic processes as coenzymes or their precursors, affecting gene regulation processes, and functioning as antioxidants.[69,85] As previously commented, some clinical laboratories are already offering patients a nutritional panel with vitamin information. Vitamin B_2, for example, is well known as a dietetically important compound that assists in the metabolism of carbohydrates, as vitamin B_6 acts as a coenzyme in many enzymatic reactions involved in the metabolism of neurotransmitters, amino acids, and lipids.[85]

The reference range for water-soluble vitamins B_2 and B_6 in blood serum are 137-370 and 2.8-74.6 μg L^{-1}, respectively, and the technique used by clinical laboratories to assess their nutritional metabolic requirements is HPLC.[85] Priego-Capote and Castro[85] described a method by CE with charge-coupled detector (CCD) and CE with photomultiplier tube (PMT) that simultaneously analyzes vitamins B_2 and B_6 within 13 min with good resolution.

Vitamin A is extremely necessary for health, so much that its deficiency can cause eye diseases such as irreversible blindness, xerophthalmia, and keratomalacia.[86,87] The reference range for vitamin A is 0.3-0.7 mg L^{-1}, and the clinical laboratories also used a method by HPLC to analyze it. Back in the 90s, Shi *et al.*[87] and Ma *et al.*[86] described methods by high performance capillary electrophoresis (HPCE), as it was called the time, in order to quantify vitamin A in microvolumes of serum and dried whole blood spots, which is important especially for neonates and low birth-weight infants.

A simultaneous determination of vitamins A and E in serum was described by Oledzka *et al.*[88] using MEKC and MEEKC methods, both with UV detection, to evaluate the nutritional status of patients with cystic fibrosis within 8 and 20 min, respectively. A deficiency of both vitamins may be associated with exocrine pancreatic insufficiency, reduced absorption, and impaired liver function. For individual tests, as it is offered by clinical laboratories using a method by HPLC (the detection is not disclosed) the reference range for vitamin E is 5-20 mg L^{-1}.[89] The proposed method by MEKC and MEKC[88] was applied to evaluate vitamin level in children suffering from cystic fibrosis within μg L^{-1} of concentration range with 97.43% accuracy for vitamin A and 95.97% for vitamin E.

To protect biocomponents from oxidative damage, since it neutralizes toxic peroxides and stabilizes free radicals, vitamin C is the most active antioxidant in many biological systems.[90,91] It is essential for the defense system and for the formation of tissues, as it participates in the constitution of the cell wall of all body cells. Just as in the determination of the other vitamins mentioned, the method used by clinical laboratories is HPLC, and the reference range for vitamin C is 0.4-2.0 mg dL^{-1}.[89] In this regard, Koh *et al.*[90] described a method by CE-UV to determine vitamin C using a stereoisomer as the internal standard within 9 min and Zinellu *et al.*[91] described another method by CE-UV that presented a good separation in less than 4 min.

All things considered, CZE-UV might be a valuable option for vitamins determination since it could offer cost-effective and faster analysis. On the other hand, the method development for some other vitamins of B complex (Figure 4) usually considered as nutritional/health markers in clinical exams may be a challenge. Low-weighted and easily ionizable ones like vitamin C, B_3, and B_6 shall be less troubling, apart from the other ones that may involve the use of elaborated electrolytes/CE modes, chromophore agents, a percentage of organic solvent and surfactants-given the hydrophobic features of some of them, but yet, with thoroughly optimization, advantageous and high-throughput methods can be achieved. Finally, about this issue, Wang *et al.*[92] published a review on a 10-year evolution of vitamin analysis by CE.

3.3. Organic/fatty acids

In both methods described by Koh *et al.*[90] and Zinellu *et al.*,[91] uric acid was also determined. Its plasma concentration can be helpful in the diagnosis of hyperuricemias, such as those found in gout, in renal failure, neoplasias, leukemias, lymphomas, myeloma, and polycythemia.[91] The reference range for uric acid is 3.4-7.0 mg dL^{-1} for men and 2.4-5.7 mg dL^{-1} for women.[89] Under optimized conditions, Sotgia *et al.*[93] succeeded in developing a method capable to determine uric acid within 1 min by CZE-UV.

Regarding the renal system, low concentrations of citric acid in the body may lead to the formation of kidney stones since this substance binds to calcium in order to prevent the precipitation of crystals. Beyond

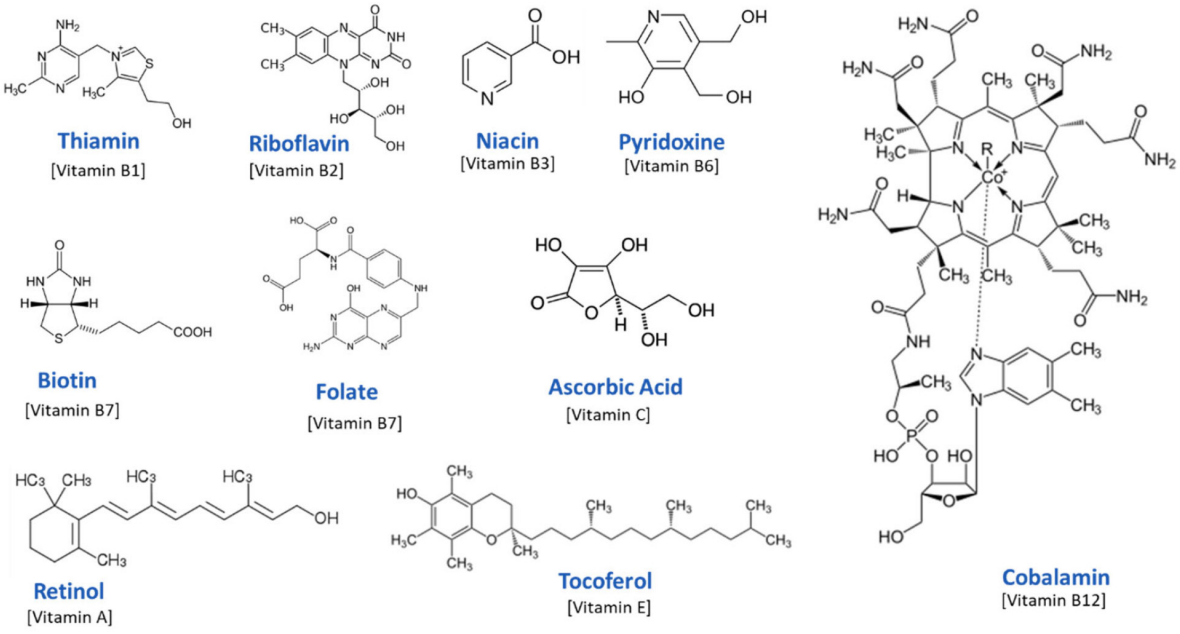


Figure 4. Chemical structure of main vitamins used as biomarkers of clinical exams for nutritional assessment and vitamin-deficiency disorders.

that, solutions containing citrate ions are administered at patients on hemodialysis to provide anticoagulation, which is an indispensable condition for renal replacement therapy. Therefore, the concentration of citric acid needs to be monitored due to the risks such as the level of ionized calcium, and the electrolyte balance of the patient.[94] A method used by the clinical laboratories is molecular absorption spectrometry, and the reference range for citric acid is 0.96-2.50 mg dL^{-1}. Considering the simplicity, rapidity, and low sample and reagent consumption, Polyakova *et al.*[94] described a method by CZE-UV for the determination of citrate ions in blood plasma within 11 min that is suitable for routine analysis.

Produced by cell metabolism, lactic acid is the final product of anaerobic glycolysis. Its serum concentration usually increase due to an inadequate amount of oxygen in cells and tissues and may indicate severe dehydration, heart and respiratory failure, hemorrhage, ketoacidosis, severe infections, shock, and liver disease. The quantification of this acid is often performed during clinical exercise testing as well as during performance testing of athletes.[95] In this regard, Wan *et al.*[37] developed a method by CE-C^4D for the analysis of lactic acid from a drop of blood within less than 5 min in order to monitor this substance increase in blood plasma during incremental cycling exercise. Usually, clinical laboratories used a colorimetric method to determine lactic acid, and its reference range is 6.3-18.9 mg dL^{-1}.[89]

Fatty acids (FAs) as health biomarkers are also worth mentioning. Essential FAs play a crucial role in inflammatory balance, cell fluidity and signaling, and more.[64,96] The "omega FAs," have been associated to cell aging, Alzheimer's, rheumatoid arthritis, cancer, and cardiovascular diseases.[96] As the omics studies advance, more on that is being elucidated. As being single-carboxylic acid with long hydrophobic carbonic chains, with or without unsaturated bonds, they can be analyzed by CZE-UV, indirectly or directly, with solvents and surfactants that make it possible their mobility through the capillary towards the detection side.

To exemplify, a study developed in our group by Barra *et al.*[97] applied a CZE-UV method for the determination of stearic acid (C18:0), elaidic acid (C18:1 *trans* 9), and oleic acid (C18:1, *cis* 9) in rat liver, which indicated the proposed method as a promising alternative for blood analysis (Figure 5). The standard technique for analysis of FA is gas chromatography with flame ionization detector (GC-FID), and so, it usually takes 60-min or longer runs, high demand of organic solvents and gas, and it generates a comprehensive set of result that may not even be necessary. In that specific case, the selectivity and eco-friendly possibility of CE is a real advantage. A thoroughly discussion of fatty acids driven methods is found in the 2014 review by de Oliveira *et al.*[98]

3.4. Hormones

Closing up the main biomarkers investigated in blood, the thyroid hormones are essential for many physiological processes.[99] Among them, thyroxine (T_4) stands out due to its importance in maintaining the normal function of multiple organ systems in adults, the normal development

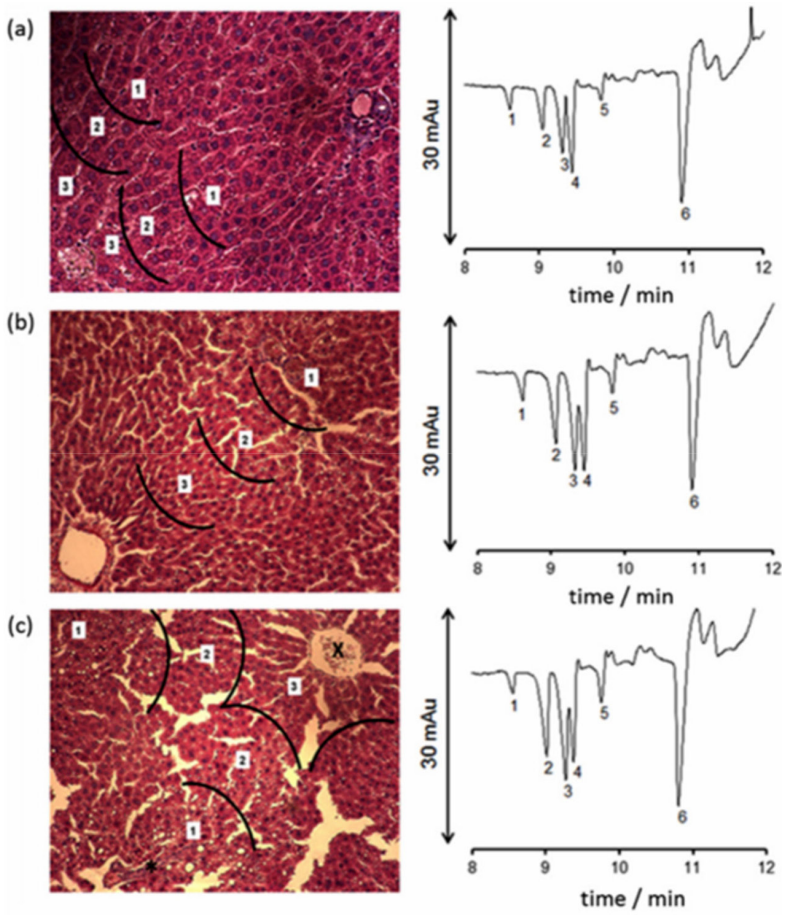


Figure 5. Fatty acid electropherograms from rat liver. Histological assessment on the left. (1) C18:0; (2) C18:1c; (3) C16:0; (4) C18:2cc; (5) C16:1c; (6) C13:0 (internal standard). Optimized electrophoretic conditions describe in Table 1 (figure from Barra *et al.*[97]).

of the central nervous system in infants, and the skeletal growth. Thyroid-stimulating hormone (TSH) secretion also plays an important role in controlling thyroid gland function and circulating levels of these thyroid hormones. A thyroid hormone deficiency can cause hypothyroidism, leading to sluggishness, depression, constipation, and weight gain. On the other hand, the excess of these hormones can cause hyperthyroidism, which involves symptoms such as heat intolerance, weight loss, diarrhea, and an enlarged thyroid gland.[100]

The aforementioned disorders are diagnosed by measuring T_4 and TSH levels, and for a more accurate diagnosis the measurement of triiodothyronine (T_3) is also useful. Regardless, the dosage of this hormones is part of the traditional full hemogram offered by clinical laboratories. Usually, analytical procedures based on immunoassays provide the reference range for T_4, TSH, and T_3 that is 4.5-12.0 μg dL^{-1}, 0.45-4.5 mIU L^{-1}, and 70-200 ng dL^{-1}, or chemiluminescence,[70] where the reference range is 5.1-14.1 μg dL^{-1}, 0.38-5.33 μIU mL^{-1}, and 0.40-2.04 ng mL^{-1}, respectively. Being IU (international units) associated to enzymatic activity.

Mu *et al.*[101] developed a method by CE with chemiluminescence detector (CL) to determine T_4 in serum within 8 min as a potential application in rapid primary diagnosis of diseases such as hypothyroid and hyperthyroid. Meanwhile, Li *et al.*[99] opted for a method by ion pair hollow fiber liquid-liquid-liquid microextration (IP-HF-LLLME) with CE-UV applied to simultaneous determination of six thyroid hormones, such as T_3 and T_4 within 13 min, while a simultaneous determination of T_3, T_4 and TSH within 3.2 min was described by Woo *et al.*[100] by capillary electrophoresis with laser induced fluorescence (CE-LIF).

Two other crucial hormones are estriol and progesterone. Estriol is formed from maternal and fetal precursors by several enzymes in the placenta or fetus in pregnancy. Thus, its measurement might be useful for the investigation and biochemical monitoring of the function of the maternal/fetus unit. Progesterone also plays an important role in women health, helping their body to prepare for conception and pregnancy, in addition to regulating the monthly menstrual cycle. So, monitoring this hormone in human serum is important for evaluating the reproductive system

health and for investigating the mechanism of various steroid prophylactics and anti-early-pregnancy drugs.[102,103]

The most common method used to quantify these hormones in clinical laboratories is the immunoassay method.[82] So, aiming at methods that present more advantages, Su *et al.*[102] described a method by capillary electrophoretic immunoassay (CEIA) for the determination of estriol in pregnant women's serum, and for the determination of progesterone, Ye *et al.*[103] described a method by microchip electrophoresis-chemiluminescence detection (MCE-CL) within 2 min that was successfully used for the determination of this hormone in serum from normal and pregnant women. Other than blood, saliva has been a stand-out bodily fluid for hormone determination to access other perspectives. The methods discussed in this section are also listed in Table 1 in Overview section.

4. Saliva

Saliva is a fluid present in the oral cavity, consisting of "a clear, slightly acidic mucoserous exocrine secretion" with normal pH ranging from 6 to 7.[104] It originates mainly from the parotid, submandibular, and sublingual salivary glands, and also from other smaller glands and the gingival crevicular fluid. The secretion of the glands presents different ionic and protein concentrations.[104,105] More than 99% of saliva consists of water. Its components include electrolytes such as sodium, potassium, calcium, magnesium, bicarbonate, and phosphate, immunoglobulins such as immunoglobulin A (IgA), proteins, enzymes, mucin, and nitrogen products (urea and ammonia). It also features other elements from leftover food, bacteria, and gum inflammation. Some of these are diffused in the blood plasma by different processes.[104,105]

The main functions of saliva consist of lubrication and protection of oral tissues, buffering and cleaning actions, in which bicarbonate is the most important buffering, maintenance of tooth integrity, antibacterial activity by immunological and non-immunological agents, tasting, and digestion. All this to maintain oral health and an adequate ecological balance.[104,106] Therefore, saliva has been used in research aimed at diagnosing, treating, and preventing oral and systemic diseases.

Recently, it is being considered the bodily fluid for monitoring the performance of athletes. The fact that saliva collection is simple, safe, and non-invasive makes its analysis interesting.[107,108] In 2008, the term "Salivaomics" was proposed, including genomics, proteomics, metabolomics, transcriptomics, and microRNA (miRNA) based on saliva samples. These analyses reflect the research pursuit on saliva constituents and the discovery of biomarkers.[109] Regarding current clinical exams, saliva analyses are related to hormonal status, neurological status, nutritional influences, metabolic influences, dental and viral diseases, among others.[106] Saliva is already used as a biological sample in some laboratory tests, such as cortisol, melatonin, testosterone, progesterone, dehydroepiandrosterone (DHEA), estriol, estradiol, estrone, iodine, IgA-secretory and amylase.[110-114]

Thus, it is clear that saliva has been used for steroid hormone dosage. This is because there is a connection between the plasma and the salivary glands, so that the hormones present in the plasma can be measured in the saliva.[105] However, only unbound hormones, i.e., the hormone-free fraction present in plasma, can diffuse into saliva and can be measured. Analytical methods for these hormones (Figure 6) include electrochemiluminescence, radioimmunoassay, and enzyme immunoassays. LC-MS/MS also appears as a possibility to measure cortisol (which is also measured by electrochemiluminescence).[114]

Overall, steroid hormones are fat-soluble compounds with conjugated bonds that indicate CE methods may

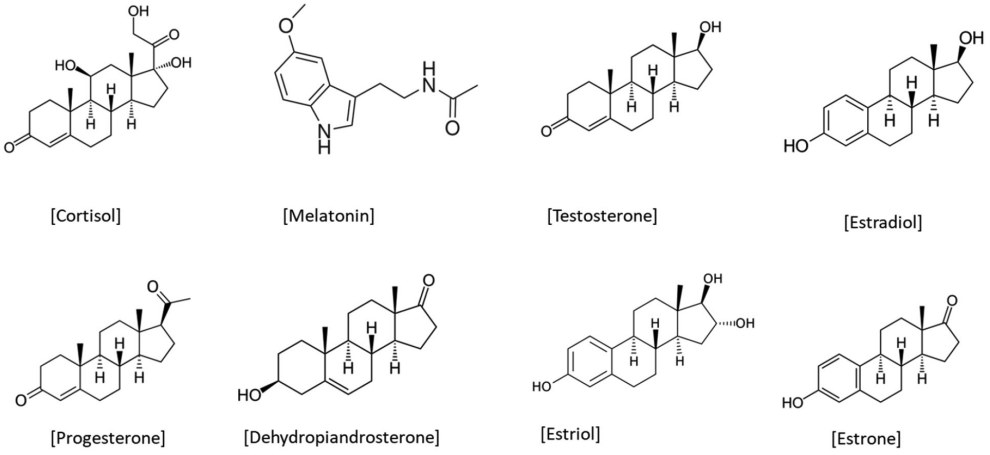


Figure 6. Chemical structure of the main steroid hormone measured in clinical laboratories.

consider non-aqueous solutions with surfactants. Ionization may be a problem, so the use of MEKC or similar modes shall be considered. In our previous work,[16] we discussed the study that consider a buffer solution with β-cyclodextrin for some of the steroids mentioned for urine analysis (Table 1). Protocol as such can be adapted to saliva samples, however, we believe that pre-concentration procedures would be required (for CE or LC approaches).

The measurement of salivary iodine allows the evaluation of iodine in the thyroid, helping in the diagnosis of diseases such as goiter and hypothyroidism, among others. The challenge for the development of an alternative salivary diagnostic approach is related to the lower concentrations of analytes in this matrix compared to other biological fluids, such as blood and urine.[107]

In this context, no reports were found for the use of electromigration techniques in the determination of iodine in saliva. It requires the use of more sensitive techniques with lower limits of detection, such as ICP as presented by Novo *et al.*[115] They developed an ICP-MS method to determine iodine and bromine in human saliva at ultra-trace concentrations.

Nevertheless, there are reports of the determination of iodide in saliva using electromigration techniques. Three papers present the use of capillary isotachophoresis (CITP) for the determination of iodide and other inorganic anions in human saliva using conductivity[116] and UV detectors.[117,118] In one of these approaches, an electrokinetic injection, also called field-amplified sample injection, was applied to increase sensitivity, and a strong counter-electroosmotic flow was necessary to allow anions migration into the capillary towards the detector. The authors emphasize that is necessary to use the standard addition method to reduce the matrix effects, associated with lower sample dilution and shorter injection time.[118]

In another work, a capillary ion electrophoresis method with UV detection was developed for the determination of iodide and other four inorganic anions in human saliva, using a polyvinyl alcohol chemically coated capillary, and a BGE composed of hexamethonium dichloride, ion-pair reagent.[119] This combination allowed the manipulation of the electrophoretic mobility of the anions, improving the resolutions between peaks, and direct injection, without any preconcentration step.

For the determination of salivary hormones, only one work was founded using an electromigration technique. A chip-based capillary electrophoresis method for simultaneous determination of testosterone, luteinizing hormone (LH), TSH, and follicle-stimulating hormone (FSH) in different biological fluids, including saliva, using LIF detection (Figure 7).[120] The saliva sample preparation involves an immunoextraction with analyte-specific antibodies immobilization on the chip, which enhances the sensitivity of the analysis, achieving concentrations in the order of pg mL^{-1}. As we can see, salivary analysis by CE is a promising field for diagnosis but needs more studies.

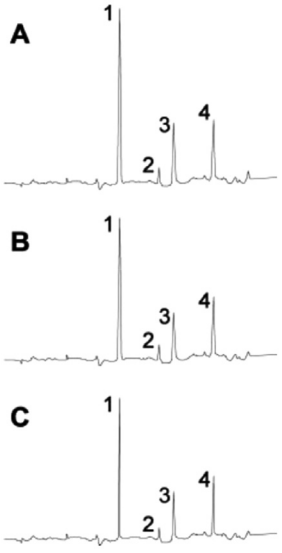


Figure 7. Electropherogram of the analysis of a few hormones in whole blood (A), saliva (B), and urine (C). (1) TSH, (2) testosterone, (3) LH, (4) FSH. Optimized experimental conditions described in Table 1 (reproduced from Wellner and Kalish[120] with copyright permission 2008 from John Wiley and Sons).

5. Stool

Contrary to what many people think, the stool is not just a waste to be thrown away. Several clinical tests can be performed to aid in the diagnosis of disease and access the patient's health. Stool examinations are recommended for patients with symptoms of a gastrointestinal infection, such as prolonged diarrhea, abdominal pain (cramps), nausea and vomiting, and stools with blood and mucus.[121] The main advantage of stool is a non-invasive collection method, even though many patients still experience embarrassment when collecting a fecal sample. Another advantage of stool examination is a lower operational cost, with moderate tests sensitivity and specificity.[122]

In the clinical laboratory, the stool should be macroscopically checked in terms of color, consistency, quantity, shape, odor, and abnormal bloody mucus.[123] The stool analysis may also include microscopic examination to determine the presence of leukocytes and fecal parasites - intestinal protozoa and/or helminths.[124] Stool cultures can help to identify the type of harmful bacteria[121] such as the Gram-positive bacteria *Staphylococcus aureus*, *Clostridium* spp., and *Bacillus cereus*; and the Gram-negatives *Vibrio* spp., *Salmonella* spp., and

Campylobacter jejuni. In addition, other diarrhea-causing microorganisms can also be detected, such as the rota-, astro-, and coronaviruses.[125] Immunological and chemical methods are available to evaluate occult blood, fat, sugars (reducing substances), pH, pancreatic enzymes, alpha-1 antitrypsin, and calprotectin.[123]

The stool has a particularity when compared to other biological samples since it carries numerous biochemical information derived from the host, its diet and gut microbiota.[126] Currently, it is already known that intestinal homeostasis depends on the combination of balanced diet and a healthy microbiota, and that an imbalance in these systems leads to chronic inflammation and metabolic dysfunction.[127,128] The human intestinal tract harbors more than 100 trillion microbial cells and its metabolic activity can influence the acquisition and development of host diseases.[127,129] In the same way, diets poor in fiber and high in fat and sugar consumption have been associated with the decreased fecal levels of short chain fatty acids (SCFAs).[128] These SCFAs are exclusively produced by resident beneficial bacteria and are important for proper gut barrier functioning.[130]

In this context, CE is an ecofriendly technique and a robust tool for diagnostic applications suitable for detecting important changes in the metabolic profile of fecal samples. From experience, fecal samples provide less troubled analysis regarding interferences, depending on the group of biomarkers on focus, which contributes for the simpler use of CZE-UV. Garcia *et al.*[131] developed a CZE-UV method to determine acetic acid, propionic acid, fumaric acid, and butyric acid. In order to investigate the functioning of the large intestine, Hodek and Krızek[132] have also developed a CZE-UV method to quantify acetic acid, propionic acid, isobutyric acid, and butyric acid and applied in human and canine fecal samples (Figure 8).

Pham *et al.*[133] investigated and optimized the separation parameters, especially the BGE composition, to separate short and medium-chain fatty acids since they are found in the intestinal microbiota and are biomarkers of physiological processes and inflammatory conditions in the organism. A CZE method with indirect UV detection was developed and used in rat fecal spiked samples to determine octanoic acid, heptanoic acid, hexanoic acid, valeric acid, isovaleric acid, butyric acid, isobutyric acid, propionic acid, and acetic acid.

Another approach was presented by Corradini *et al.*[134] and Matsumoto *et al*.[135] They developed CE methods to analyze *Bifidobacterium* strains from fecal culture. Corradini *et al.*[134] work was based on a CZE-UV method adopting a co-electroosmotic flow approach and separate sulfate (from the minimal synthetic medium), malonic acid

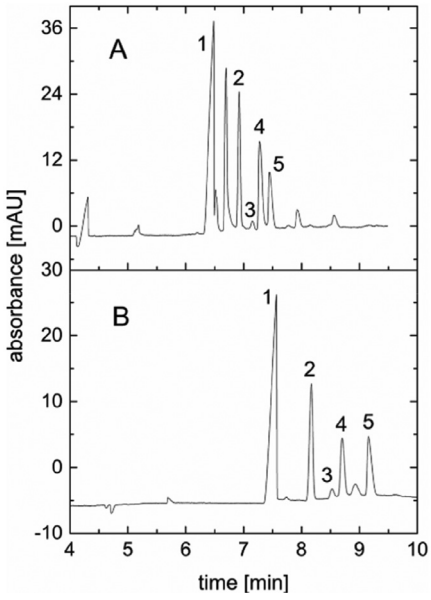


Figure 8. Electropherograms of SCFA in human (A) and canine (B) feces. (1) Acetic acid; (2) propionic acid; (3) iso-butyric acid; (4) butyric acid. Optimized experimental conditions described at Table 1 (reproduced from Hodek and Křížek[132] with copyright permission 2019 from Royal Society of Chemistry).

(internal standard (IS)), succinic acid, acetic acid, and lactic acid. Matsumoto *et al*.[135] investigated the effects of the probiotic *Bifidobacterium animalis* subsp lactis LKM512 and the expression of metabolites in fecal samples, for this was used a CE coupled with time-of-flight mass spectrometry (TOF-MS).

The human metabolome database (HMDB)[136] has a huge number of small molecule metabolites of human origin. More than 6,700 metabolites have already been described in stool and have been cataloged in this electronic database. Several sub-classes of organic compounds can be detected and quantified in stool, for example fatty acids, carboxylic acids and conjugates (butyric acid, propionic acid, formic acid, and isobutyric acid), dicarboxylic acids and derivatives (fumaric acid), alcohols and polyols (ethanol), benzenediols (dopamine), cholestane steroids (cholesterol), bilirubins (bilirrubin), and pyrimidines and pyrimidine derivatives (thymine). The evaluation of these and other metabolites in stool can be of great value for the diagnosis and control of diseases. Although urine and blood are often used to investigate metabolites, stool samples show great potential for it.

Fecal metabolite profiles hold a huge potential for fecal biomarker discovery. Metabolic changes were found in patients with several diseases and disorders, such as colorectal cancer, inflammatory bowel disease (Crohn's disease and ulcerative colitis), food allergies (celiac disease), depression, Alzheimer's disease, and Autism Spectrum Disorder.[126,130] Furthermore, the combined

analysis of serum and stool biomarkers has been suggested to increase precision and improve the diagnosis.[137]

CE can fill this gap and be used for diagnostic analysis in fecal samples to search for analytes that can be problematic by chromatographic techniques, such as amino acids, peptides, organics acids, amines, nucleosides, in addition to the use of samples with high saline content and cell culture media.[138] Together with all the procedures mentioned at the previous sections, the methods are detailed in Table 1 in Overview section.

6. Sweat

Sweat is a biofluid produced by the sweat glands placed in mammals under the skin and it is composed of water, mostly, with electrolytes, sugar, and hormones. Sweat acts like a thermoregulator for the body helping it to keep its internal temperature stable, although other functions are also attributed to sweat like immune defense of the skin and excretion. The former allows sweat testing to be a viable source of information about the organism metabolism and its general state.[139]

Sweat-based testing is known for being a non-invasive method with low relative cost, besides, it is an abundant fluid with harmless stimulation. The methods of acquiring sweat samples for testing have evolved significantly from the conventional body-wash-down technique, where the individual is subjected to perform exercises on an ergonomic bike in a cardboard chamber, then his body is rinsed with deionized water and further collected. This method demands time and it is nonpractical since it is limited to laboratory environments, thereby making it difficult to application in the field or on routine tests.[140] Better ways of stimulating sweat production include wearable devices with the premise of replacing time-consuming and expensive lab tests. Those devices also allow continuous monitoring of biomarkers, i.e., a complete mapping of the signs during the day.[141]

Some metabolites can be monitored by sweat with the goal of finding evidence of drugs abuse to clinical diagnostics.[142-144] Even though sweat does not have the same preference as other biofluids for diagnostics purposes, there are several applications for sweat testing, like chloride monitoring for the diagnosis of cystic fibrosis. The gold standard for cystic fibrosis is the Gibson and Cook technique which includes sweat stimulation by pilocarpine iontophoresis followed by manual titration or quantitative colorimetric test. That kind of test requires skilled analysts and is time-consuming, therefore, capillary electrophoresis can be a viable option for the chloride determination on sweat.[145,146]

Dubot *et al.*[147] used CE-C^{4}D to determine chloride in sweat samples for the diagnosis of cystic fibrosis with the sample preparation followed the Gibson and Cook procedure. The developed method has interesting parameters such as 2 μL of sample required and 2-min-long run, including the pre-conditioning. The economic amount of sample for the analysis added to a high analytical frequency makes this method a viable option for routine analysis. The colorimetric test was used as a comparative reference. Dubot's method resulted in zero false positives and zero false negatives in 55 samples with a correlation between the methods of 0.993. Agreement between both methods was assessed and confirmed by statistical tests including Passing Bablock regression and Bland and Altman plots, concluding that both methods have the same capacity for giving cystic fibrosis auxiliary diagnostics.

In 2017, Ďurč[148] proposed a method by double opposite end injection capillary electrophoresis with contactless conductivity detection (DOEI-CE-C^{4}D) to determine chloride concentration in sweat samples, similar as the one method commented on the Urine section. In this case, a simple swipe with a swab moistened with deionized water was used to collect the sample. Gibson-Cook may be uncomfortable and takes about 30 min to be performed. The experiment was conducted for 30 individuals, 10 adults and 10 pediatrics already diagnosticated with cystic fibrosis and 10 healthy individuals for control purposes. The CE method was applied to skin swipe samples but also to the standard Gibson-Cook samples. Full separation among the ions specimen was achieved and the method was validated.

For diagnostics motivation was used the ratio (Cl^-/K^+). Lower ratio was found in healthy individuals than in the ones with cystic fibrosis. Principal component analysis was performed for (Cl^-/K^+); (Cl^-/Na^+); (Na^+/K^+) as variables and a cluster for healthy individuals was observed. To compare the diagnostic potential, the content of chloride determined by DOEI-CE-C^{4}D was evaluated against the standard coulometric method resulting in a correlation of 0.9972. However, the absolute measure for the skin swipe test resulted in a much lower content of chloride detected what can cause misclassification on the diagnostics. The (Cl^-/K^+) ratio was proposed as a marker, but further evaluation is necessary.

Staying on cystic fibrosis diagnosis, more recently Macedo *et al.*[149] developed a method using MSI-CE-MS (Figure 9) to find other biomarkers but chloride, since the intermediate level of chloride in sweat is 30-59 mmol L^{-1} is known for bringing inconclusive diagnostic results. Sweat samples for newborns less than 3 months old with positive and negative diagnostics for cystic fibrosis were analyzed. As a result of the screening protocol, pilocarpic

acid, L-asparagine (Asn), phthalic acid, and L-glutamine were found to be particularly significant to differentiate positive and negative for cystic fibrosis. The method itself, focused on the screening of amino acids and organic acids, together with the one MSI-based protocol mentioned in the previous section, is an interesting perspective on how multisegmented injection is a good strategy to enhance analytical frequency. Although the mentioned MSI-CE-MS is considered high-resolution MS, MSI-CE alone can be adapted to other bioanalysis considering simpler CE setups.

Another common use of sweat testing is monitoring electrolyte loss during physical activities, sodium being the one with the bigger interest. The mapping of electrolytes loss allows their reposition during physical activity which helps to increase athletes' performance.[150] The classical methods for sodium sweat analysis include ion chromatography, flame photometry and ion-selective electrodes. Alternatively, a CE-UV method was developed by Hirokawa *et al.*[151] using citric acid as a complexing agent and it was able to determine inorganic cations standards. In the same method, organic compounds including diethanolamine, triethanolamine, ornithine, histidine, lysine, arginine were also detected. The linearity and limit of detection were calculated for every species ranging from 0.2 µL for arginine and lysine to 1.7 µL for Na^+. The method was validated and applied to sweat samples for 3 individuals. Differentiation between the content of sweat from the finger and the forearm was also made, resulting in a higher concentration found in the finger-extracted samples.

Other sweat metabolites were also analyzed by CZE without diagnostic premises, pyruvate related to muscle fatigue[152] and taurine (a non-proteinogenic amino acid) are some examples. Sweat analysis has also been made with other separation techniques as GC[153-155] and LC.[156] The use of CE for sweat analysis is summarized in Table 1 in Overview section. Typically, sweat testing offers a variety of benefits when compared to blood or urine analyses, such as the reduced potential for sample tampering, longer periods of detection in certain instances, and less invasiveness compared to blood samples, besides, the low sample amount required for the analysis make CZE a good fit for further studies. Depending on what it is being searched and metabolic response, sweat might be a good way of assessing. Even though the potential of sweat as biofluid for clinical

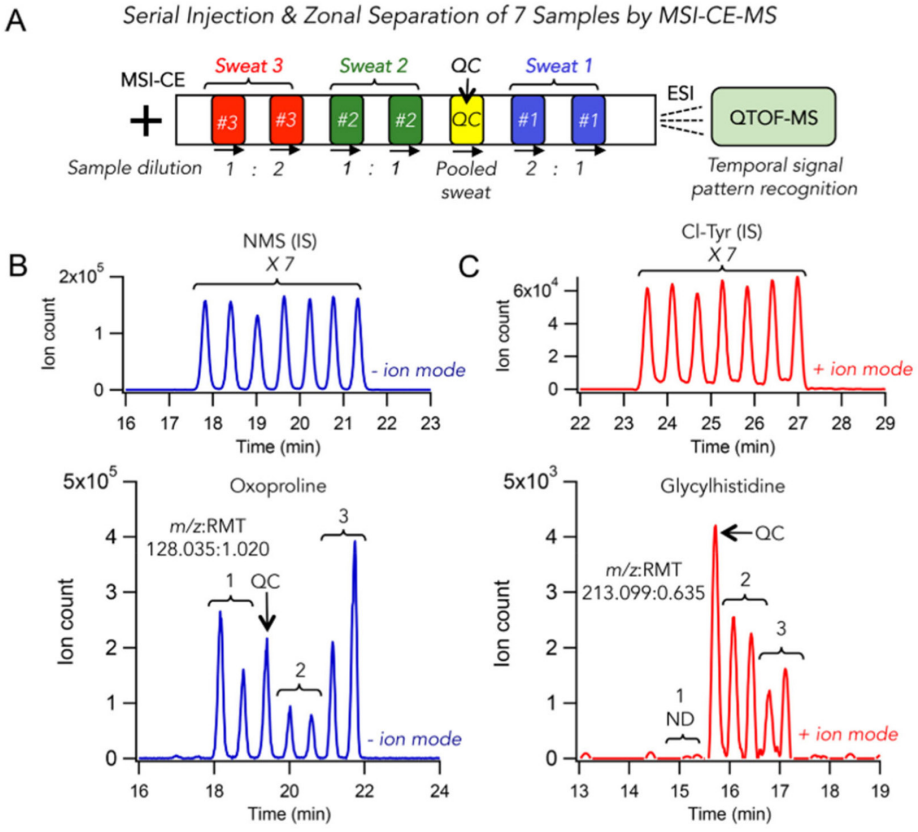


Figure 9. Metabolite profiling and analytical quality control (QC) by MSI-CE-MS. (A) Serial injection scheme. (B) Representation of an amino acid EIE (extracted ion electropherogram) detected at negative ionization mode (oxoproline, *m/z* 128.0352) and (C) representation of an amino acid EIE detected at positive ionization mode (glycylhistidine, *m/z* 213.0990). ND: not detected; NMS: sodium 2-naphthalenesulfonate; Cl-Tyr: 3-chloro-L-tyrosine; RMT: relative migration time. *m/z*: mass-to-charge ratio. Optimized electrophoretic condition described at Table 1 (reproduced from Macedo *et al.*[149] with copyright permission 2017 from the American Chemical Society).

diagnostics is pretty much untapped when compared to the traditional ones, sweat analysis, especially by CZE, is a promising yet challenging line of research.

7. Overview

The commented compilation of existing exams brought attention to a series of biomarkers of specific chemical groups that are already being offered to individuals as a possibility for health assessment. As a matter effect, the same marker can be measured in different bodily fluids (Figure 10) which is, from an analytical perspective, an interesting advantage. Table 1 summarizes all the methods commented throughout the sections. Since the same analyte can be measured in more than one bodily fluid, a comprehensive comparative overview of methods is described. Overall, the capillary electrophoresis can offer in some cases just the advantages that are currently being searched by physicians. That being said, as the precision medicine evolves, the analytical procedures shall go along with it by acting as a reliable tool to new measurements.

Regarding the analysis of complex matrices as bodily fluids, among all the advantages commented throughout the work, sample preparation is also one of them. By using a separation technique as CE, some of the laborious reactions required in colorimetric analysis widely used in laboratory exams, are no longer necessary. Plus, as CE includes analysis through unpackaged columns as the fused silica tubes, steps that involve syringe filters and dilution with organic solvents are not a priority. In most cases considering CE, the "dilute-and-shoot" protocol is very acceptable, which means that the sample is submitted to protein precipitation and/or dilution only. For other analysis, in which low concentrations may be a problem or when extraction is necessary, as commented by Armenta *et al.*[157] liquid-liquid extraction (LLE) or solid-phase extraction (SPE) are the most popular nowadays, with the possibility of using smart materials that agreed with the Green Chemistry perspective. On this issue, some thoroughly written reviews[158,159] cover the applicability, when required, of the sample preparation protocols used for CE analysis.

Whole blood and blood parts (serum and plasma), urine and feces are bodily matrices knowingly common in clinical exam routines, thereby being a good start to adapt some tests to CE alternatives in those samples. As saliva is now being considered for a series of investigations, and sweat testing is evolving from only intensive cardio assessment to other analysis, other non-invasive samples may be introduced as possibilities, also considering CE. For example, some toxicological screens in hair are already performed. Analysis such as those could also be performed by CE and associations.[160-162] Some studies that considered tears as main sample have also shown promising outcomes[163-166] that can evolve to new eye-care assays in the future.

Altogether, years of academic studies may have the information needed for the implementation of the technique in clinical laboratories or other medical facilities. In this review, we discussed some of the options found in the specialized literature in a study of case that hopefully helps the technique to take a turn into a day-to-day instrumentation that would help give patients and physicians just the answer they need.

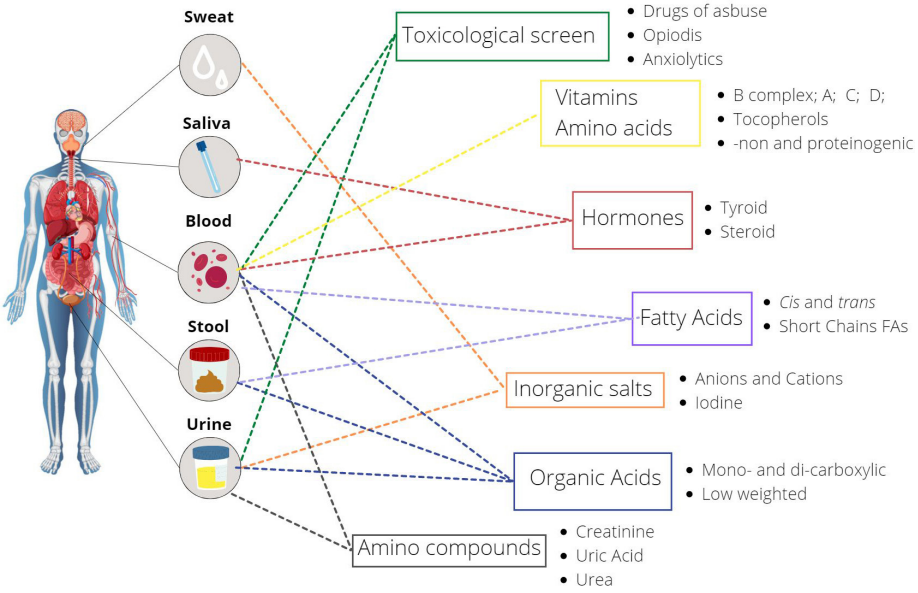


Figure 10. Schematic map of the main group of biochemicals set as biomarker for health assessment as auxiliary diagnostic.

Table 1. Summary of CE methods that can be adapted for analysis of the bodily fluids discussed

Suggested clinical application	Analyte	Analytical platform	CE experimental conditions	Detector parameters	Reference
Electrolyte balance; inorganic salts and minerals screening; cystic fibrosis	Cl^-, SO_4^{2-}, SO_2^-, NO_3^-, NH_4^+, K^+, Na^+, Mg^{2+}, Ca^{2+}	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} histidine (pH 3.6); capillary: 25 µm i.d. × 20 cm for cations, 15 cm for anions; 3.45 kPa × 2 min, 30 kV, 25 °C	low pass: 2 Hz, frequency: 800 kHz	44
	NH_4^+, K^+, Na^+, Mg^{2+}, Ca^{2+}	CZE- UV	BGE: 7.5 mmol L^{-1} Cu^{II} acetate, 15 mmol L^{-1} ethylenediamine, 2 mmol L^{-1} triethanolamine (pH 8); capillary: 75 µm i.d. × 50 cm, 3.43 kPa, 25 kV, 25 °C	230 nm	167
	Cl^-, SO_4^{2-}, NO_3^-, NO_2^-, HCO_3^-	CZE-UV/ CZE- C^4D	BGE: 1.0 mol L^{-1} AA 12 mmol L^{-1} L-histidine, and 2 mmol L^{-1} 18-crown-6-ether, capillary: 75 µm i.d. × 65 cm; 50 mbar × 5 min, 15 kV, 25 °C	C^4D excitation: 80 V, frequency: 1000 kHz; 200 nm	46
	Cl^-, SO_4^{2-}, NO_3^-, NO_2^-, HCO_3^-	CZE-UV	BGE: 60 mmol L^{-1} CrO_4^{2-}, 2.5 mmol L^{-1} CTAB, 0.875% (v/v) ACN, and 2.625% (v/v) MeOH; capillary: 75 µm i.d. × 40 cm; –5 kV, 25 mbar × 5 s	254 nm	47
	NO_3^-, NO_2^-	CZE-UV	BGE: 100 mmol L^{-1} TRIS/HCl buffer and 0.15 mmol L^{-1} CTAB at (pH 8.2); capillary: 75 µm i.d. × 40 cm; 50 mbar × 10 s; –15 kV, 25 °C	210 nm	168
	Cl^-	DOEI-CE-C^4D	BGE: 20 mmol L^{-1} 2-(*N*-morpholino)ethanesulfonic acid, 20 mmol L^{-1} L-histidine, and 6 mmol L^{-1} 18-crown-6 (pH 6), capillary: 50 µm i.d. × 40 cm; +15 kV; 10 cm × 6 s (sample) + 10 cm × 6 s (BGE)	custom made C^4D - frequency: 1.8 MHz	148
Kidney activity, urolithiasis, gout	creatinine	CZE-UV	BGE: 500 mmol L^{-1} formic acid (pH 2.03), capillary 50 µm i.d. × 61.5 cm, 50 mbar × 10 s; current fixed at 39 µA; 25 °C	200 nm	58
		CZE-UV	BGE: 80 mmol L^{-1}, phosphate buffer ($H_3PO_4/H_2PO_4^-$) (pH 2.3), capillary: 75 µm i.d. × 8.5 cm; 25 °C, SEIP: 15 kV; 15 mbar, 4 s sample; 15 mbar, 4 s IS; 10 mbar, 2 s BGE	200 nm	56
	uric acid	CZE-UV	BGE: 75 mmol L^{-1} sodium borate glycylglycine solution titrated with 5 mol L^{-1} NaOH (pH 9); uncoated fused-silica capillary, 50 µm i.d. × 60 cm; –28 kV, 15 ºC	292 nm	93
Aminoacidpathies	Cys and Hcy	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% (v/v) PEG-600; undeactivated silica capillary of 50 µm i.d. × 32 cm; 50 mbar × 45 s (sample), followed by –17 kV × 30 s (0.5 M KOH); –17 kV, 30 ºC	285 nm	79
	Hcy, Cys, methionine (MT), and glutamic acid (GA)	CE-MS/MS	BGE: 5 mol L^{-1} acetic acid; fused silica capillary 50 µm i.d. × 60 cm; 50 mbar × 10 s; 25 kV; 20 °C, SHL: 5 mmol L^{-1} acetic acid in methanol/water (50:50 v/v) at 6 µL min^{-1}, 160 °C; 6 L min^{-1}; 4500 V	MRM; *m/z* transitions: HCy 136-90; Cys 122-76; MT 150-104; GA 148-84	80
	20 proteinogenic amino acids	CE-MS/MS	BGE: 500 mmol L^{-1} formic acid; SHL: methanol/water (50:50 v/v) with 5 mmol L^{-1} NH_4Ac at 8 µL min^{-1}; 10 psi; 300 °C; 10 L min^{-1}; 4000 V; fragmentor: 100 V	MRM	67
	amino acid and organic acid screening	MSI-CE-MS	BGE: 1 mol L^{-1} formic acid with 15% ACN (pH 1.8) for cations; 50 mmol L^{-1} ammonium bicrbonate (pH 8.5) for cations; fused silica capillary, 50 µm i.d. and 120 cm; 50 mbar × 5 s (sample), 40 s (BGE); 30 kV; 25 °C; SHL (ESI+): 0.1% (v/v) formic acid in 60:40 (v/v) methanol:water at 10 µL min^{-1}; SHL (ESI–): 50:50 (v/v) methanol:water at 10 µL min^{-1}; 300 °C; 16 L min^{-1}; 8 psi; sheath gas 200 °C; 3.5 L min^{-1}; capillary and nozzle voltage 2000 V; fragmentor: 380 V	full scan 50-1700 *m/z*	169
Vitamins screening	B_2 and B_6	CZE-CCD	BGE: 30 mmol L^{-1} KH_2PO_4 (pH 8.5) adjusted with formic acid; fused-silica capillary, 75 µm i.d. × 58 cm; 10 s, –25 kV, 35 ºC	495 nm	85
	A	CZE-LIF	BGE: 50 mmol L^{-1} Na_2HPO_4, (pH 7.8) adjust with 1.5 mol L^{-1} phosphoric acid, fused-silica capillary, 50 µm i.d. and 60 cm total length; 10 s, –20 kV	325 nm; 465 nm	86

Table 1. Summary of CE methods that can be adapted for analysis of the bodily fluids discussed (cont.)

Suggested clinical application	Analyte	Analytical platform	CE experimental conditions	Detector parameters	Reference
Vitamins screening	A and E	MEKC	BGE: 35 mmol L^{-1} SDS, 100 mmol L^{-1} boric acid, and 5 mmol L^{-1} sodium tetraborate (pH 7.75); capillary: 75 µm i.d. × 57 cm; 10 s, –18 kV, 22 ºC	280 nm	88
		MEEKC	BGE: mixture of propan-2-ol (16.2 g), butan-1-ol (6.6 g), *n*-octane (0.8 g), SDS (2.883 g), and 100 mmol L^{-1} sodium tetraborate; capillary: 50 µm i.d. and 47 cm; 34.5 mbar × 7 s, –18 kV, 22 ºC	200 nm	88
	C	CE-UV	BGE: 100 mmol L^{-1} sodium borate (pH 8); capillary: 75 µm i.d. and 60.2 cm; 34.5 mbar × 7 s, –30 kV, 25 ºC	264 nm	91
Hormones determination	T_4, T_3 and TSH	CE-LIF	BGE: 25 mmol L^{-1} $Na_2B_4O_7$-NaOH (pH 9.3); capillary: 75 µm i.d, × 21 cm; 5 cm × 5 s, –371 V/C	543 nm; 635 nm	100
	T_4 and T_3	LLLME-CE-UV	BGE: 25 mmol L^{-1} phosphate buffer (pH 2.15) containing 10% (v/v) ACN and 0.5% (m/v) of PEG 10000; capillary: 75 µm i.d. × 64.5 cm; 50 mbar × 5 s, –20 kV, 25 ºC	214 nm	99
	estriol	CEIA-LIF	BGE: 125 mmol L^{-1} TRIS-borate (pH 7.5) containing 0.99 mg mL^{-1} hydrogel; fused-silica capillary, 75 µm i.d. and 57 cmf 50 mbar × 5 s, –22 kV, 20 ºC	488 nm	102
	TSH, testosterone, LH, FSH	ICE-LIF	BGE: 100 mmol L^{-1} phosphate buffer (pH 7.5); PEEK capillary: 50 µm i.d.	633 nm	120
Drugs of abuse assessment	opioids, stimulants, and benzodiazepines	MSI-CE-MS	BGE: 1 mol L^{-1} formic acid with 13% ACN (pH 1.8), fused silica capillary, 75 µm i.d. and 120 cm, 50 mbar × 5 s (sample), 40 s (BGE), 30 kV, 25 °C, SHL: 0.1% (v/v) formic acid in 60:40 (v/v) methanol:water at 6 µL min^{-1}, 200 °C; 16 L min^{-1}; 8 psi; sheath gas 200 °C; 3.5 L min^{-1}, capillary and nozzle voltage 2000 V; 380 V fragmentor	full scan 50-1700 *m/z*	68
Fatty acid assessment	C16:0 C18:0, C18:1 9c, C18:2cc, C16:1c, C18:3ccc	CZE-UV	BGE: 15.0 mmol L^{-1} (NaH_2PO_4/Na_2HPO_4) pH 6.86, 4.0 mmol L^{-1} SDBS, 8.3 mmol L^{-1} Brij 35, 45% v/v of ACN and 2.1% v/v of 1-octanol, TSH capillary 75 µm i.d. and 48.5 cm, 12.5 mbar 5 s; 19 kV; 25 °C	224 nm	97
Modulation of intestinal functions	AA, PA, FU, and BA	CZE-UV	BGE: 234 mmol L^{-1} phosphate buffer (pH 6.10) and 12% methanol; capillary: 50 µm i.d. and 37 cm; 33 mbar × 20 s, –10 kV, 25 ºC	200 nm	131
	AA, PA, IBA, and BA	CZE-UV	BGE: 10 mmol L^{-1} β-Ala + 10 mM benzoic acid + 0.005% polybrene + 4 mM α-CD; capillary: 75 µm i.d. and 80 cm; 50 mbar × 5 s, –20 kV, 25 ºC	350 nm	132
	OA, HepA, HexA, VA, IVA, BA, IBA, PA, AA	CZE-UV	BGE: 100 mmol L^{-1} borate buffer, 7 mM adenosine 5'-monophosphate, 5 mM α-CD (pH 6.5), 7.5% methanol. capillary: 75 µm i.d. and 56 cm, 5 mbar × 50 s, 30 kV, 25 ºC	320 nm	133

AA: acetic acid; ACN: acetonitrile; BA: butyric acid; BGE: background electrolyte; C4D: capacitively-coupled contactless conductivity detector; CCD: charge-coupled detector; CE: capillary electrophoresis; CEIA: capillary electrophoretic immunoassay; CTAB: cetyltrimethylammonium bromide; CZE: capillary zone electrophoresis; Cys: cysteine; DDAPS: dodecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate; DOEI: double opposite end injection; ESI: electrospray ionization; FSH: follicle-stimulating hormone; FU: fumaric acid; HCl: hydrochloric acid; Hcy: homocysteine; HepA: heptanoic acid; HexA: hexanoic acid; HPCZE: high performance capillary zone electrophoresis; IBA: isobutyric acid; ICE: immunoaffinity capillary electrophoresis; i.d: internal diameter; IVA: isovaleric acid; IS: internal standard; KOH: potassium hydroxide; LH: luteinizing hormone; LIF: laser induced fluorescence; LLLME: liquid-liquidliquid microextration; MEKC: micellar electrokinetic chromatography; MEEKC: microemulsion electrokinetic chromatography; MeOH: methanol; MS: mass spectrometry; MS/MS: tandem mass spectrometry; MSI: multisegmented injection; MRM: multiple reaction monitoring; NaOH: sodium hydroxide; NDS: 1,5-naphthalenedisulfonic acid disodium salt hydrate; NTS: 1,3(6,7)-naphthalenetrisulfonic acid trisodium salt hydrate; OFM: osmotic flow modifier; OA: octanoic acid; PA: propionic acid; PEEK: polyetheretherketone; PEG-600: polyethylene glycol 600; SEIP: short-end injection program; SDBS: sodium dodecylbenzenesulfonate; SDS: sodium docecyl sulfate; SHL: sheath liquid; T_3: triiodothyronine; T_4: thyroxine; TEA: triethanolamine; TRIS: tris(hydroxymethyl)aminomethane; TSH: thyroid-stimulating hormone; TSP: standard polyimide coating; UV: ultraviolet; VA: valeric acid; α-CD: alpha-cyclodextrin; β-Ala: beta-alanina.

Acknowledgments

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).

Author Contributions

Olívia B. de Oliveira Moreira was responsible for conceptualization, data curation, investigation, visualization, writing original draft, writing-review and editing; Bruna M. Berlande for conceptualization, data curation, investigation, visualization, writing original draft; Jéssica C. Queiroz de Souza for conceptualization, data curation, investigation, visualization, writing original draft; João Marcos B. Candido for conceptualization, data curation, investigation, visualization, writing original draft; Lauren H. Jaeger for conceptualization, data curation, investigation, visualization, writing original draft; Luiz Henrique C.Adriano for conceptualization, data curation, investigation, visualization, writing original draft; Marco Antônio D. Assad e Brito for conceptualization, data curation, investigation, visualization, writing original draft; Maria Patrícia do Nascimento for conceptualization, data curation, investigation, visualization, writing original draft; Patrícia A. Geraldo for conceptualization, data curation, investigation, visualization, writing original draft; Paula R. Chellini for conceptualization, data curation, investigation, visualization, writing original draft; Marcone Augusto L. de Oliveira for conceptualization, funding acquisition, project administration, resources, visualization, writing original draft, writing-review and editing.

Olívia B. O. Moreira *has a bachelor degree in Industrial Chemistry (2017) from Fluminense Federal University, a Master's degree in Chemistry (2020) and is currently a PhD student in Analytical Chemistry both in the Federal University of Juiz de Fora at the Analytical Chemistry and Chemometrics Group (GQAQ). She has experience with development, optimization, and validation of analytical methods, metabolomics, design of experiments and multiplatform instrumentation (LC-MS/MS; CE-DAD; CE-MS and spectroscopy). Recently, she is working in the development of new diagnostic assays for infectious diseases.*

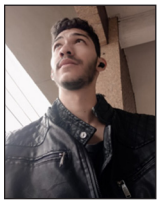
Bruna M. Berlande *is undergraduate student majoring in Chemistry at the Federal University of Juiz de Fora and is a member of the Analytical Chemistry and Chemometrics Group (GQAQ) as a part of the scientific initiation scholarship program. Currently, she is developing analytical methods by capillary electrophoresis for determination of antioxidants in cosmetical samples.*

Jéssica C. Q. de Souza *has bachelor, licentiate, and Master's degrees in Chemistry from the Federal University of Juiz de Fora (UFJF). She is currently a PhD student in Analytical Chemistry at UFJF and is a member of the Analytical Chemistry and Chemometrics Group (GQAQ). Has experience with electromigration techniques and chromatography for pharmaceutical samples, including the development, optimization, and validation of analytical methods. She is currently working on the development of analytical methods for antimalarial drug analysis.*

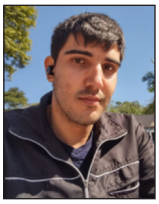
João Marcos B. Candido *has a bachelor's degree in Chemistry (2021) and is studying for a Master's degree in Chemistry, both at the Federal University of Juiz de Fora. He is currently a member of the Analytical Chemistry and Chemometrics Group (GQAQ) and has experience in the development, optimization and validation of analytical methods by electromigration techniques and the design of experiments. Recently, he is working on developing new capillary electrophoresis methods for antiviral drugs for the treatment of COVID-19.*

Lauren H. Jaeger *is BSc in Pharmacy, MSc in Tropical Medicine and PhD in Parasitology. Professor at the Department of Pharmaceutical Sciences in Federal University of Juiz de Fora and a member of Brazilian Society of Parasitology. She has experience in Parasitology, phylogeny and discovery of new drugs for the treatment of parasitic diseases.*

Luiz Henrique C. Adriano *has a bachelor's degree in Chemistry from the Federal University of Juiz*



de Fora (2020) and is currently a Master's Degree student in Analytical Chemistry at the same institution as a member of the Analytical Chemistry and Chemometrics Group (GQAQ). Recently, he is working with capillary electrophoresis methods applied to phytochemical systems with focus in Anthocyanins.



Marco Antonio D. Assad e Brito *undergraduate student majoring in Chemistry at the Federal University of Juiz de Fora and he is a member of the Analytical Chemistry and Chemometrics Group (GQAQ). He is participating as scientific initiation scholarship program in a study that develops analytical methods to diagnose infectious diseases, such as COVID-19, Zika, Chikungunya and Dengue through metabolomics in urine using capillary electrophoresis and mass spectrometry.*

Maria Patrícia do Nascimento *is a PhD student in Chemistry at the Federal University of Juiz de Fora (UFJF) and is a member of the Analytical Chemistry and Chemometrics Group (GQAQ). She has a Master's degree in Analytical Chemistry (2019) and a bachelor's and licentiate degree in Chemistry in 2015 and 2017, respectively, at the UFJF. She has experience with electromigration and chromatographic techniques for ionic liquids and biological samples and developing, optimizing, and validating analytical methods. Currently, she is working on developing analytical methods for the determination of biomarkers in biological samples.*

Patrícia A. Geraldo *has a bachelor's degree in Chemistry (2021) from the Federal University of Juiz de Fora and is a Master's Degree student in Analytical Chemistry at the same institution. She is a member of the Analytical Chemistry and Chemometrics Group (GQAQ), and is currently working on the development of analytical methods for fatty acids analysis in food and biological samples by electromigration techniques.*

Paula R. Chellini *is BSc in Pharmacy, MSc in statistics, PhD in Pharmaceutical Sciences. Professor at the Department of Pharmaceutical Sciences in Federal University of Juiz de Fora and is a member of the Analytical Chemistry and Chemistry Group (GQAQ). She has experience in analysis and control of medicine, drugs quantification in biological matrices, validation of analytical and bioanalytical methods (HPLC-UV, LC-MS/MS and CE-UV), pharmaceutical equivalence, and bioequivalence.*

Marcone Augusto L. de Oliveira *is Full Professor at the Chemistry Department at Federal University of Juiz de Fora where he coordinates the Analytical Chemistry and Chemometrics Group (GQAQ). Has experience in development and optimization of analytical methods by capillary electrophoresis (CE-UV and CE-MS), high performance liquid chromatography (HPLC-UV and HPLC-MS/MS), and gas chromatography (GC-FID and GC-MS) for applications in food analysis, pharmaceuticals (drugs, cosmetics, phytochemicals), biofuels (biodiesel), metabolomics (phytochemicals and clinical diagnostics), petroleum, environmental area, preparation of new monolithic stationary phases for CE and nano-LC, in addition to data science (experimental design, multivariate analysis and machine learning).*

References

1. Vaz, F. A. S.; de Oliveira, C. L. M.; de Oliveira, M. A. L.; *Quim. Nova* **2015**, *38*, 732. [Crossref]
2. da Silva, J. A. F.; Coltro, W. K. T.; Carrilho, E.; Tavares, M. F. M.; *Quim. Nova* **2007**, *30*, 740. [Crossref]
3. Chen, F. T. A.; Liu, C.-M.; Hsieh, Y.-Z.; Sternberg, J. C.; *Clin. Chem.* **1991**, *37*, 14. [Crossref]
4. Petersen, J. R.; Okorodudu, A. O.; Mohammad, A.; Payne, D. A.; *Clin. Chim. Acta* **2003**, *330*, 1. [Crossref]
5. Jabeen, R.; Payne, D.; Wiktorowicz, J.; Mohammad, A.; Petersen, J.; *Electrophoresis* **2006**, *27*, 2413. [Crossref]
6. Colyer, C. L.; Tang, T.; Chiem, N.; Harrison, D. J.; *Electrophoresis* **1997**, *18*, 1733. [Crossref]
7. Hage, D. S. In *Clinical Applications of Capillary Electrophoresis Methods in Molecular Biology*, vol. 1972.; Phillips, T. M., ed.; Humana: New York, NY, USA, 2009, p. 3-11. [Crossref]
8. Rudaz, S.; Tobolkina, E.; *Anal. Chem.* **2021**, *93*, 8107. [Crossref]
9. Zhang, C.; Hage, D. S.; *Clinical Chemistry Applications of Capillary Electromigration Methods*; Elsevier Inc.: Amsterdan, 2018.
10. Perrett, D.; *Ann. Clin. Biochem.* **1999**, *36*, 133. [Crossref]
11. Horká, M.; Horký, J.; Kubesová, A.; Mazanec, K.; Matoušková, H.; Lais, K.; *Analyst* **2010**, *135*, 1636. [Crossref]
12. Buszewski, B.; Kłodzińska, E.; *TrAC, Trends Anal. Chem.* **2016**, *78*, 95. [Crossref]

13. Tavares, M. F. M.; *Quim. Nova* **1997**, *20*, 493. [Crossref]
14. Amézqueta, S.; Subirats, X.; Fuguet, E.; Ràfols, C.; Rosés, M.; *Handbook of Analytical Separations*, vol. 8; Valkó, K. L., ed.; Elsevier: Amsterdan, 2020, p. 633. [Crossref]
15. Oyaert, M.; Delanghe, J.; *Ann. Lab. Med.* **2019**, *39*, 15. [Crossref]
16. 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.; *Braz. J. Anal. Chem.* **2023**, *10*, 17. [Crossref]
17. 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.; *PLoS One* **2013**, *8*, e73076. [Crossref]
18. Liao, J. C.; Churchill, B. M.; *Pediatr. Clin. North Am.* **2001**, *48*, 1425. [Crossref]
19. Delanghe, J. R.; Speeckaert, M. M.; *Clin. Biochem.* **2016**, *49*, 1346. [Crossref]
20. Simerville, J. A.; Maxted, W. C.; Pahira, J. J.; *Am. Fam. Physician* **2005**, *71*, 1153. [PubMed]
21. Kouri, T.; Fogazzi, G.; Gant, V.; Hallander, H.; Hofmann, W.; Guder, W. G.; *Scand. J. Clin. Lab. Invest.* **2000**, *60*, 1. [Crossref]
22. Piech, T. L.; Wycislo, K. L.; *Vet. Clin. North Am.: Small Anim. Pract.* **2019**, *49*, 233. [Crossref]
23. Callens, A. J.; Bartges, J. W.; *Vet. Clin. North Am.: Small Anim. Pract.* **2015**, *45*, 621. [Crossref]
24. Patel, H. P.; *Pediatr. Clin. North Am.* **2006**, *53*, 325. [Crossref]
25. Brüngel, M.; Kluthe, R.; Fürst, P.; *Ann. Nutr. Metab.* **2001**, *45*, 169. [Crossref]
26. CDC Environmental Health, *Laboratory Procedure Manual*, https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/labmethods/HULT_H_R_MET_LYTES.pdf, accessed in May 2023.
27. Labtest, *Chlorides Liquiform*, https://labtest.com.br/wp-content/uploads/2016/09/Ref_115_RevAbril2011_Ref040618_Eng.pdf, accessed in May 2023.
28. Labcorp, *Chloride, 24-Hour Urine*, https://www.labcorp.com/tests/003160/chloride-24-hour-urine, accessed in May 2023.
29. UCSF Health, *Potassium Urine Test*, https://www.ucsfhealth.org/medical-tests/potassium-urine-test#:~:text=Normal Results,of potassium in your body, accessed in May 2023.
30. Tanaka, T.; Okamura, T.; Miura, K.; Kadowaki, T.; Ueshima, H.; Nakagawa, H.; Hashimoto, T.; *J. Hum. Hypertens.* **2002**, *16*, 97. [PubMed]
31. Sherman, R. A.; Eisinger, R. P.; *JAMA, J. Am. Med. Assoc.* **1982**, *247*, 3121. [Crossref]
32. National Library of Medicine, *Nitrites in Urine*, https://medlineplus.gov/lab-tests/nitrites-in-urine/#:~:text=A test called a urinalysis,by different types of bacteria, accessed in May 2023.
33. Najeeb, S.; Munir, T.; Rehman, S.; Hafiz, A.; Gilani, M.; Latif, M.; *J. Coll. Physicians Surg. Pakistan* **2015**, *25*, 108. [PubMed]
34. Devillé, W. L. J. M.; Yzermans, J. C.; van Duijn, N. P.; Bezemer, P. D.; Van der Windt, D. A. W. N.; Bouter, L. M.; *BMC Urol.* **2004**, *14*, 4. [Crossref]
35. James, S.; Tyrrell-Price, J.; Atkinson, C.; Hunt, L.; Searle, A.; Phillips, K.; Penfold, C.; Carter, J.; Ness, A.; *Clin. Nutr. ESPEN* **2021**, *42*, 339. [Crossref]
36. Roberts, J. R.; *Emerg. Med. News* **2007**, *37*, 14. [Crossref]
37. Wan, Q. J.; Kubáň, P.; Tanyanyiwa, J.; Rainelli, A.; Hauser, P. C.; *Anal. Chim. Acta* **2004**, *525*, 11. [Crossref]
38. Freitas, C. B.; Moreira, R. C.; Tavares, M. G. O.; Coltro, W. K. T.; *Talanta* **2016**, *147*, 335. [Crossref]
39. Sidorova, A. A.; Grigoriev, A. V.; *J. Anal. Chem.* **2012**, *67*, 478. [Crossref]
40. Miyado, T.; Tanaka, Y.; Nagai, H.; Takeda, S.; Saito, K.; Fukushi, K.; Yoshida, Y.; Wakida, S. I.; Niki, E.; *J. Chromatogr. A* **2004**, *1051*, 185. [Crossref]
41. Lucas, S. B.; Duarte, L. M.; Rezende, K. C. A.; Coltro, W. K. T.; *Micromachines* **2022**, *13*, 1736. [Crossref]
42. Nussbaumer, S.; Fleury-Souverain, S.; Bouchoud, L.; Rudaz, S.; Bonnabry, P.; Veuthey, J.-L.; *J. Pharm. Biomed. Anal.* **2010**, *53*, 130. [Crossref]
43. Morcos, E.; Wiklund, N. P.; *Electrophoresis* **2001**, *22*, 2763. [Crossref]
44. Yamamoto, S.; Fujiwara, H.; Maruyama, K.; Tanaka, Y.; Kinoshita, M.; Suzuki, S.; *Anal. Sci.* **2019**, *35*, 295. [Crossref]
45. Sáiz, J.; Koenka, I. J.; Mai, T. D.; Hauser, P. C.; García-Ruiz, C.; *TrAC, Trends Anal. Chem.* **2014**, *62*, 162. [Crossref]
46. Zhao, J.; Xu, Z.; *Anal. Methods* **2021**, *13*, 801. [Crossref]
47. do Nascimento, M. P.; Marinho, M. V.; de Sousa, R. A.; de Oliveira, M. A. L.; *Anal. Methods* **2023**, *15*, 311. [Crossref]
48. Wang, Y.; Zeng, Z.; Yang, L.; Zeng, H.; Li, Y.; Pu, Q.; Zhang, M.; *Anal. Chem.* **2023**, *95*, 2146. [Crossref]
49. Tang, K. W. A.; Toh, Q. C.; Teo, B. W.; *Singapore Med. J.* **2015**, *56*, 7. [Crossref]
50. Srivastava, T.; Alon, U. S.; Althahabi, R.; Garg, U.; *Pediatr. Res.* **2009**, *65*, 113. [Crossref]
51. Delanghe, J. R.; Speeckaert, M. M.; *NDT Plus* **2011**, *4*, 83. [Crossref]
52. Saibaba, K. S. S.; Vijaya Bhaskar, M.; Srinivasa Rao, P. V. L. N.; Ramana, G. V.; Dakshinamurty, K. V.; *Indian J. Clin. Biochem.* **1998**, *13*, 55. [Crossref]
53. Labtest, *Uréia UV-Liquiform*, https://labtest.com.br/wp-content/uploads/2016/09/Ur%C3%A9ia_UV_Liquiform_104_Port.pdf, accessed in May 2023.
54. OLABS, https://amrita.olabs.edu.in/?sub=79&brch=17&sim=206&cnt=2, accessed in May 2023.
55. CDC Environmental Health, *Laboratory Procedure Manual, Uric Acid*; https://wwwn.cdc.gov/nchs/data/nhanes/2007-2008/labmethods/ur_biopro_e_met_lx20.pdf, accessed in May 2023.

56. Moreira, O. B. O.; de Souza, J. C. Q.; Candido, J. M. B.; do Nascimento, M. P.; Chellini, P. R.; de Lemos, L. M.; de Oliveira, M. A. L.; *Talanta* **2023**, *258*, 124465. [Crossref]
57. Qu, S.; Cao, Q.; Ma, J.; Jia, Q.; *Talanta* **2020**, *219*, 121280. [Crossref]
58. Piestansky, J.; Galba, J.; Kovacech, B.; Parrak, V.; Kovac, A.; Mikuš, P.; *Biomed. Chromatogr.* **2020**, *34*, e4907. [Crossref]
59. National Library of Medicine, https://pubchem.ncbi.nlm.nih.gov/compound/Uric-Acid#section=LogP, accessed in May 2023.
60. Wang, Q.; Wen, X.; Kong, J.; *Crit. Rev. Anal. Chem.* **2020**, *50*, 359. [Crossref]
61. Vernerová, A.; Kujovská Krčmová, L.; Melichar, B.; Švec, F.; *Clin. Chem. Lab. Med.* **2021**, *59*, 797. [Crossref]
62. National Library of Medicine, https://pubchem.ncbi.nlm.nih.gov/compound/Urea#section=Caco2-Permeability, accessed in May 2023.
63. Genova Diagnostics, *Toxic Element Clearance Profile Ratio to Creatinine*, https://www.gdx.net/files/downloads/NutrEval-FMV-Sample-Report.pdf, accessed in May 2023.
64. Genova Diagnostics, https://www.gdx.net/files/downloads/NutrEval-FMV-Sample-Report.pdf 2023, accessed in May 2023.
65. Genova Diagnostics, *Metabolomix*+, https://www.gdx.net/products/metabolomix, accessed in May 2023.
66. Genova Diagnostics, *Amino Acids Analysis, Urine*, https://www.gdx.net/core/sample-reports/amino-acids-urine-3500-sample-report.pdf, accessed in May 2023.
67. Piestansky, J.; Olesova, D.; Galba, J.; Marakova, K.; Parrak, V.; Secnik, P.; Secnik, P.; Kovacech, B.; Kovac, A.; Zelinkova, Z.; Mikus, P.; *Molecules* **2019**, *24*, 3345. [Crossref]
68. Dibattista, A.; Rampersaud, D.; Lee, H.; Kim, M.; Britz-Mckibbin, P.; *Anal. Chem.* **2017**, *89*, 11853. [Crossref]
69. Voet, D.; Voet, J. G.; Pratt, C. W.; *Fundamentos de Bioquímica*, 4th ed.; Artmed: Porto Alegre, 2014.
70. Burtis, C. A.; Burtis, D. E.; *Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics*; Elsevier: St. Louis, Missouri, USA, 2015.
71. Vannucchi, H.; Melo, S. S.; *Arq. Bras. Endocrinol. Metabol.* **2009**, *53*, 540. [Crossref]
72. Ridker, P. M.; Manson, J. E.; Buring, J. E.; Shih, J.; Matias, M.; Charles, H.; Hennekens, M.; *JAMA, J. Am. Med. Assoc.* **1999**, *281*, 1817. [Crossref]
73. Kamat, P. K.; Vacek, J. C.; Kalani, A.; Tyagi, N.; *Open Neurol. J.* **2015**, *9*, 9. [Crossref]
74. Demuth, K.; Ducros, V.; Michelsohn, S.; Paul, J. L.; *Clin. Chim. Acta* **2004**, *349*, 113. [Crossref]
75. Barbosa, T. M. C. C.; Carvalho, M. G.; Silveira, J. N.; Rios, J. G.; Komatsuzaki, F.; Carvalho Godói, L.; Costa, G. H. Y.; *J. Bras. Patol. Med. Lab.* **2014**, *50*, 402. [Crossref]
76. La'ulu, S. L.; Rawlins, M. L.; Pfeiffer, C. M.; Zhang, M.; Roberts, W. L.; *Am. J. Clin. Pathol.* **2008**, *130*, 969. [Crossref]
77. Gascón, T. M.; Schindler, F.; de Oliveira, C. G. B.; Souza, F. I. S.; Hix, S.; Sarni, R. O. S.; Bacan, A. P. A.; D'Almeida, V.; del Giglio, A.; Fonseca, F. L. A.; *Einstein* **2010**, *8*, 187. [Crossref]
78. Ta, H. Y.; Collin, F.; Perquis, L.; Poinsot, V.; Ong-Meang, V.; Couderc, F.; *Anal. Chim. Acta* **2021**, *1174*, 338233. [Crossref]
79. Ivanov, A. V.; Bulgakova, P. O.; Virus, E. D.; Kruglova, M. P.; Alexandrin, V. V.; Gadieva, V. A.; Luzyanin, B. P.; Kushlinskii, N. E.; Fedoseev, A. N.; Kubatiev, A. A.; *Electrophoresis* **2017**, *38*, 2646. [Crossref]
80. Cieslarova, Z.; Lopes, F. S.; do Lago, C. L.; França, M. C.; Simionato, A. V. C.; *Talanta* **2017**, *170*, 63. [Crossref]
81. Miyaki, K.; Zeng, H. L.; Nakagama, T.; Uchiyama, K.; *J. Chromatogr. A* **2007**, *1166*, 201. [Crossref]
82. Rasmussen, K.; Moller, J.; *Ann. Clin. Biochem.* **2000**, *37*, 627. [Crossref]
83. Amores-Sánchez, M. I.; Medina, M. Á.; *Clin. Chem. Lab. Med.* **2000**, *38*, 199. [Crossref]
84. Ubbink, J. B.; *Semin. Thromb. Hemost.* **2000**, *26*, 233. [Crossref]
85. Priego-Capote, F.; de Castro, M. D. L.; *Electrophoresis* **2005**, *26*, 2376. [Crossref]
86. Ma, Y.; Wu, Z.; Furr, H. C.; Lammi-Keefe, C.; Craft, N. E.; *J. Chromatogr. B: Biomed. Sci. Appl.* **1993**, *616*, 31. [Crossref]
87. Shi, H.; Ma, Y.; Humphrey, J. H.; Craft, N. E.; *J. Chromatogr. B: Biomed. Sci. Appl.* **1995**, *665*, 89. [Crossref]
88. Oledzka, I.; Kaźmierska, K.; Plenis, A.; Kamińska, B.; Baczek, T.; *J. Pharm. Biomed. Anal.* **2015**, *102*, 45. [Crossref]
89. Fleury Group, https://www.fleury.com.br/exames/acido-lactico-plasma, accessed in May 2023.
90. Koh, E. V.; Bissell, M. G.; Ito, R. K.; *J. Chromatogr. A* **1993**, *633*, 245. [Crossref]
91. Zinellu, A.; Carru, C.; Sotgia, S.; Deiana, L.; *Anal. Biochem.* **2004**, *330*, 298. [Crossref]
92. Wang, X.; Li, K.; Yao, L.; Wang, C.; van Schepdael, A.; *J. Pharm. Biomed. Anal.* **2018**, *147*, 278. [Crossref]
93. Sotgia, S.; Carru, C.; Scanu, B.; Pisanu, E.; Sanna, M.; Pinna, G. A.; Gaspa, L.; Deiana, L.; Zinellu, A.; *Anal. Bioanal. Chem.* **2009**, *395*, 2577. [Crossref]
94. Polyakova, E. V.; Shuvaeva, O. V.; Borisov, A. S.; *J. Anal. Chem.* **2018**, *73*, 906. [Crossref]
95. Kubáň, P.; Dosedělová, V.; Martma, K.; Rannama, I.; Reinpold, K.; Shimmo, R.; *Molecules* **2021**, *26*, 5817. [Crossref]
96. Genova Diagnostics, *NutrEval*, https://www.gdx.net/core/support-guides/NutrEval-Support_guide.pdf, accessed in May 2023
97. Barra, P. M. C.; Oliveira, P. L.; Aragão, D. M. O.; Sabarense, C. M.; Aarestrup, B. J. V.; Azevedo, M. S.; Costa, A. C. O.; Micke, G. A.; Oliveira, M. A. L.; *J. Braz. Chem. Soc.* **2014**, *25*, 675. [Crossref]
98. 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.; *Molecules* **2014**, *19*, 14094. [Crossref]

99. Li, P.; Hu, B.; He, M.; Chen, B.; *J. Chromatogr. A* **2014**, *1356*, 23. [Crossref]
100. Woo, N.; Kim, S. K.; Kang, S. H.; *J. Chromatogr. A* **2017**, *1509*, 153. [Crossref]
101. Mu, X.; Li, S.; Lu, X.; Zhao, S.; *Electrophoresis* **2014**, *35*, 962. [Crossref]
102. Su, P.; Zhang, X. X.; Wang, Y. C.; Chang, W. B.; *Talanta* **2003**, *60*, 969. [Crossref]
103. Ye, F.; Liu, J.; Huang, Y.; Li, S.; Zhao, S.; *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2013**, *936*, 74. [Crossref]
104. Humphrey, S. P.; Williamson, R. T.; *J. Prosthet. Dent.* **2001**, *85*, 162. [Crossref]
105. Nunes, L. A. S.; de Macedo, D. V.; *J. Bras. Patol. Med. Lab.* **2013**, *49*, 247. [Crossref]
106. Thamaraiselvi, V. T.; Brundha, M. P.; *EJMCM* **2020**, *7*, 2914.
107. Cui, Y.; Yang, M.; Zhu, J.; Zhang, H.; Duan, Z.; Wang, S.; Liao, Z.; Liu, W.; *Med. Nov. Technol. Devices* **2022**, *13*, 100115. [Crossref]
108. Zhang, C.-Z.; Cheng, X.-Q.; Li, J.-Y.; Zhang, P.; Yi, P.; Xu, X.; Zhou, X.-D.; *Int. J. Oral Sci.* **2016**, *8*, 133. [Crossref]
109. Papale, F.; Santonocito, S.; Polizzi, A.; Giudice, A. Lo; Capodiferro, S.; Favia, G.; Isola, G.; *Metabolites* **2022**, *12*, 638. [Crossref]
110. Fleury Group, *Manual de Exames*, https://www.fleury.com.br/medico/exames/cortisol-saliva, accessed in May 2023.
111. DB diagnósticos, https://gde.diagnosticosdobrasil.com.br/GDE_Home/DetalheDoExame.aspx?ValorReferenciaId=55933999&ExameId=CORTS, accessed in May 2023.
112. LabVitrus, *Estriol Livre Salivar*, https://www.labvitrus.com.br/exame-de-rotina/estriol-livre-salivar/23, accessed in May 2023.
113. Lemos Laboratórios, *Dosagem de Iodo*, https://lemoslab.com.br/produtos_exame/dosagem-de-iodo/, accessed in May 2023.
114. de Oliveira, A. P. R. S.; de Carvalho, C. O.; Brandão, I. P.; *Rev. Bras. Análises Clínicas* **2021**, *53*, 32. [Crossref]
115. Novo, D. L. R.; Mello, J. E.; Rondan, F. S.; Henn, A. S.; Mello, P. A.; Mesko, M. F.; *Talanta* **2019**, *191*, 415. [Crossref]
116. Sádecká, J.; Polonský, J.; *Talanta* **2003**, *59*, 643. [Crossref]
117. Xu, Z.; Doi, T.; Timerbaev, A. R.; Hirokawa, T.; *Talanta* **2008**, *77*, 278. [Crossref]
118. Ren, H.; Qiu, H.; Liang, X.; Wang, X.; Jiang, S.; *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2013**, *935*, 75. [Crossref]
119. Mori, M.; Yamamoto, T.; Kaseda, M.; Yamada, S.; Itabashi, H.; *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2012**, *887-888*, 1. [Crossref]
120. Wellner, E. F.; Kalish, H.; *Electrophoresis* **2008**, *29*, 3477. [Crossref]
121. Garedaghi, Y.; *Int. J. Med. Parasitol. Epidemiol. Sci.* **2020**, *1*, 25. [Crossref]
122. Soares, F. A.; Benitez, A. D. N.; dos Santos, B. M.; Loiola, S. H. N.; Rosa, S. L.; Nagata, W. B.; Inácio, S. V.; Suzuki, C. T. N.; Bresciani, K. D. S.; Falcão, A. X.; Gomes, J. F.; *Rev. Soc. Bras. Med. Trop.* **2020**, *53*, e20190535. [Crossref]
123. Kasırga, E.; *Turk Pediatr. Ars.* **2019**, *54*, 141. [Crossref]
124. Ngwese, M. M.; Manouana, G. P.; Moure, P. A. N.; Ramharter, M.; Esen, M.; Adégnika, A. A.; *Trop. Med. Infect. Dis.* **2020**, *5*, 93. [Crossref]
125. Kotgire Santosh, A.; *J. Clin. Diagnostic Res.* **2012**, *6*, 503. [Crossref]
126. Gratton, J.; Phetcharaburanin, J.; Mullish, B. H.; Williams, H. R. T.; Thursz, M.; Nicholson, J. K.; Holmes, E.; Marchesi, J. R.; Li, J. V.; *Anal. Chem.* **2016**, *88*, 4661. [Crossref]
127. Qin, J.; Li, R.; Raes, J.; Arumugam, M.; Burgdorf, K. S.; Manichanh, C.; Nielsen, T.; Pons, N.; Levenez, F.; Yamada, T.; Mende, D. R.; Li, J.; Xu, J.; Li, S.; Li, D.; Cao, J.; Wang, B.; Liang, H.; Zheng, H.; Xie, Y.; Tap, J.; Lepage, P.; Bertalan, M.; Batto, J. M.; Hansen, T.; Le Paslier, D.; Linneberg, A.; Nielsen, H. B.; Pelletier, E.; Renault, P.; Sicheritz-Ponten, T.; Turner, K.; Zhu, H.; Yu, C.; Li, S.; Jian, M.; Zhou, Y.; Li, Y.; Zhang, X.; Li, S.; Qin, N.; Yang, H.; Wang, J.; Brunak, S.; Doré, J.; Guarner, F.; Kristiansen, K.; Pedersen, O.; Parkhill, J.; Weissenbach, J.; Bork, P.; Ehrlich, S. D.; Wang, J.; Antolin, M.; Artiguenave, F.; Blottiere, H.; Borruel, N.; Bruls, T.; Casellas, F.; Chervaux, C.; Cultrone, A.; Delorme, C.; Denariaz, G.; Dervyn, R.; Forte, M.; Friss, C.; Van De Guchte, M.; Guedon, E.; Haimet, F.; Jamet, A.; Juste, C.; Kaci, G.; Kleerebezem, M.; Knol, J.; Kristensen, M.; Layec, S.; Le Roux, K.; Leclerc, M.; Maguin, E.; Melo Minardi, R.; Oozeer, R.; Rescigno, M.; Sanchez, N.; Tims, S.; Torrejon, T.; Varela, E.; De Vos, W.; Winogradsky, Y.; Zoetendal, E.; *Nature* **2010**, *464*, 59. [Crossref]
128. Venegas, D. P.; De La Fuente, M. K.; Landskron, G.; González, M. J.; Quera, R.; Dijkstra, G.; Harmsen, H. J. M.; Faber, K. N.; Hermoso, M. A.; *Front. Immunol.* **2019**, *10*, 277. [Crossref]
129. Nicholson, J. K.; Holmes, E.; Kinross, J.; Burcelin, R.; Gibson, G.; Jia, W.; Pettersson, S.; *Science* **2012**, *336*, 1262. [Crossref]
130. Jones, J.; Reinke, S. N.; Ali, A.; Palmer, D. J.; Christophersen, C. T.; *Sci. Rep.* **2021**, *11*, 13964. [Crossref]
131. Garcia, A.; Olmo, B.; Lopez-Gonzalvez, A.; Cornejo, L.; Rupérez, F. J.; Barbas, C.; *J. Pharm. Biomed. Anal.* **2008**, *46*, 356. [Crossref]
132. Hodek, O.; Křížek, T.; *Anal. Methods* **2019**, *11*, 4575. [Crossref]
133. Pham, U.; Alvarado, L.; Suess, G. J.; Shamsi, S. A.; Frantz, K.; *Electrophoresis* **2021**, *42*, 1914. [Crossref]
134. Corradini, C.; Bianchi, F.; Matteuzzi, D.; Amoretti, A.; Rossi, M.; Zanoni, S.; *J. Chromatogr. A* **2004**, *1054*, 165. [Crossref]
135. Matsumoto, M.; Ebata, T.; Hirooka, J.; Hosoya, R.; Inoue, N.; Itami, S.; Tsuji, K.; Yaginuma, T.; Muramatsu, K.; Nakamura, A.; Fujita, A.; Nagakura, T.; *Ann. Allergy, Asthma, Immunol.* **2014**, *113*, 209. [Crossref]
136. The human metabolome database, https://hmdb.ca/, accessed in May 2023.
137. Wilkens, R.; Dolinger, M.; Burisch, J.; Maaser, C.; *Gastroenterology* **2022**, *162*, 1476. [Crossref]

138. García, A.; Godzien, J.; Gonzálvez, Á. L.-; Barbas, C.; *Bio* **2017**, *9*, 99. [Crossref]
139. Ibrahim, N. F. A.; Sabani, N.; Johari, S.; Manaf, A. A.; Wahab, A. A.; Zakaria, Z.; Noor, A. M.; *Sensors* **2022**, *22*, 7670. [Crossref]
140. Montagu, A.; *JAMA* **1962**, *17*, 179. [PubMed]
141. Xu, J.; Fang, Y.; Chen, J.; *Biosensors* **2021**, *11*, 245. [Crossref]
142. Lozano, J.; García-Algar, O.; Vall, O.; de la Torre, R.; Scaravelli, G.; Pichini, S.; *Ther. Drug Monit.* **2007**, *29*, 711. [Crossref]
143. de la Torre, R.; Pichini, S.; *Clin. Chem.* **2004**, *50*, 1961. [Crossref]
144. Gallardo, E.; Queiroz, J. A.; *Biomed. Chromatogr.* **2008**, *288*, 278. [Crossref]
145. Servidoni, M. F.; Gomez, C. C. S.; Marson, F. A. L.; Toro, A. A. D. C.; Ribeiro, M. Â. G. O.; Ribeiro, J. D.; Ribeiro, A. F.; *J. Bras. Pneumol.* **2017**, *43*, 121. [Crossref]
146. Mishra, A.; Greaves, R.; Massie, J.; *Clin. Biochem. Rev.* **2005**, *26*, 135. [Crossref]
147. Dubot, P.; Liang, J.; Dubs, J.; Missiak, Y.; Sarazin, C.; Couderc, F.; Caussé, E.; *Pract. Lab. Med.* **2019**, *13*, e00114. [Crossref]
148. Ďurč, P.; Foret, F.; Pokojová, E.; Homola, L.; Skřičková, J.; Herout, V.; Dastych, M.; Vinohradská, H.; Kubáň, P.; *Anal. Bioanal. Chem.* **2017**, *409*, 3507. [Crossref]
149. Macedo, A. N.; Mathiaparanam, S.; Brick, L.; Keenan, K.; Gonska, T.; Pedder, L.; Hill, S.; Britz-McKibbin, P.; *ACS Cent. Sci.* **2017**, *3*, 904. [Crossref]
150. Baker, L. B.; *Sports Med.* **2017**, *47*, 111. [Crossref]
151. Hirokawa, T.; Okamoto, H.; Gosyo, Y.; Tsuda, T.; Timerbaev, A. R.; *Anal. Chim. Acta* **2007**, *581*, 83. [Crossref]
152. Jin, M.; Dong, Q.; Dong, R.; Jin, W.; *Electrophoresis* **2001**, *22*, 2793. [Crossref]
153. Brunet, B. R.; Barnes, A. J.; Choo, R. E.; Mura, P.; Jones, H. E.; Huestis, M. A.; *Ther. Drug Monit.* **2010**, *32*, 40. [Crossref]
154. Brunet, B. R.; Barnes, A. J.; Scheidweiler, K. B.; Mura, P.; Huestis, M. A.; *Anal. Bioanal. Chem.* **2008**, *392*, 115. [Crossref]
155. Fucci, N.; de Giovanni, N.; *Ther. Drug Monit.* **2007**, *29*, 452. [Crossref]
156. Schneider, S.; Ait-m-bark, Z.; Schummer, C.; Lemmer, P.; Yegles, M.; Appenzeller, B.; Wennig, R.; *Anal. Toxicol.* **2008**, *32*, 260. [Crossref]
157. Armenta, S.; Esteve-Turrillas, F. A.; Garrigues, S.; de la Guardia, M.; *Sustainable Chem. Pharm.* **2021**, *21*, 100411. [Crossref]
158. Kim, J.; Choi, K.; Chung, D. S. In *Chemistry, Molecular Sciences and Chemical Engineering*, vol. 3; Elsevier: Amsterdam, 2012, p. 701. [Crossref]
159. Álvarez-Sánchez, B.; Priego-Capote, F.; Luque de Castro, M. D.; *TrAC, Trends Anal. Chem.* **2010**, *29*, 111. [Crossref]
160. Gottardo, R.; Fanigliulo, A.; Bortolotti, F.; De Paoli, G.; Pascali, J. P.; Tagliaro, F.; *J. Chromatogr. A* **2007**, *1159*, 190. [Crossref]
161. Gottardo, R.; Bortolotti, F.; De Paoli, G.; Pascali, J. P.; Mikšík, I.; Tagliaro, F.; *J. Chromatogr. A* **2007**, *1159*, 185. [Crossref]
162. Tagliaro, F.; Manetto, G.; Crivellente, F.; Scarcella, D.; Marigo, M.; *Forensic Sci. Int.* **1998**, *92*, 201. [Crossref]
163. V enkata Saijyothi, A.; Angayarkanni, N.; Syama, C.; Utpal, T.; Shweta, A.; Bhaskar, S.; Geetha, I. K.; Vinay, P. S.; Thennarasu, M.; Sivakumar, R. M.; Prema, P.; *Proteomics Clin. Appl.* **2011**, *5*, 206. [Crossref]
164. Du, F.; Zhao, W.; Cao, S.; Fung, Y. S.; *J. Sep. Sci.* **2015**, *38*, 3271. [Crossref]
165. Chadwick, R. R.; Hsieh, J. C.; Resham, K. S.; Nelson, R. B.; *J. Chromatogr. A* **2002**, *671*, 403. [Crossref]
166. Georgakopoulos, C. D.; Lamari, F. N.; Karathanasopoulou, I. N.; Gartaganis, V. S.; Pharmakakis, N. M.; Karamanos, N. K.; *Biomed. Chromatogr.* **2010**, *24*, 852. [Crossref]
167. Olsšauskaite, V.; Paliulionyte, V.; Padarauskas, A.; *Clin. Chim. Acta* **2000**, *293*, 181. [Crossref]
168. de Oliveira, M. A. L.; Soares, D. C.; Tostes, G. S.; Guimarães, M. C.; Vaz, F. A. S.; *Am. J. Anal. Chem.* **2012**, *3*, 484. [Crossref]
169. de Macedo, A. N.; Macri, J.; Hudecki, P. L.; Saoi, M.; McQueen, M. J.; Britz-McKibbin, P.; *J. Appl. Lab. Med.* **2017**, *1*, 649. [Crossref]

Submitted: March 8, 2023
Published online: June 13, 2023