

REVIEW

# Capillary electrophoresis mass spectrometry based metabolomics

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**Capillary electrophoresis–mass spectrometry (CE-MS) is a powerful orthogonal technique capable of filling in gaps in the identification, quantitation and isomeric resolution of many small hydrophilic and charged metabolites. The metabolome is a large complex mixture of molecules for which not one technique nor a combination of techniques can optimally identify and measure it in its entirety. LC-MS, GC-MS and NMR have been the widely used for metabolomics for the past 20 years for a wide range of applications, each technique having shown uniqueness and advantages, for specific applications or target metabolic chemical space. CE-MS captures a unique metabolic chemical space beyond these standard methods providing another window into metabolomics profiling. This review will focus on the recent publications published within 2016 focusing on biotechnology and pharmaceutical applications of CE-MS.**

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**Keywords:** capillary electrophoresis mass spectrometry, metabolomics, metabolite profiling.

## Introduction

Since its first formal definition more than a decade ago, metabolomics, or, the comprehensive analysis of all metabolites present within a biological system, has attracted growing interest in clinical research by academia, industry and government labs. This is most prevalent in biomarker and drug development applications where a considerable change has been witnessed in how new diagnoses, prognoses, and therapeutic options are being discovered and developed using omic technologies. Moreover, many chronic diseases suggest a strong metabolic involvement or even a clear metabolic cause, including cancer. Together with the other omic disciplines, including genomics and proteomics, metabolomics plays a key role in the implementation of personalized medicine; evidence-based medicine designed for individually designed healthcare strategies. In turn, biomarker discovery and the understanding of biochemical pathways typically rely on a

multimodal approach. Among these modalities, there continues to be a growing interest in CE-MS based development and implementation in clinical development.

## Formulating the problem

Depending on how you classify the metabolome, there is a complex chemical space separated by hydrophilicity, polarity and size, excluding a broad range of metabolites classified as lipids, separately referred to as lipidomics. This class of metabolites also covers a large range of physical properties for which specifically designed platforms work the best. For example, for years liquid chromatography-mass spectrometry (LC-MS) has been used to capture a host of hydrophilic and hydrophobic metabolites, while gas chromatography-mass spectrometry (GC-MS) has been used to capture small molecular weight metabolites. For this review, the metabolome refers to endogenous molecules with a molecular weight typically less than 1000 Kd.

To measure, catalogue, and compare the entirety of the metabolic space, the implementation of comprehensive mass spectral databases was needed (e.g., Human Metab-

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olome Database (HMDB), METLIN, MassBank, LIPID MAPS, LipidBlast, NIST 14). These databases have helped drive the field and enable untargeted discovery by CE-MS.

The HMDB, for example, lists thousands of metabolites, their physical properties and, for many common metabolites, metabolite concentrations in various biological matrices (blood, urine, saliva, CSF). While METLIN reports structure and mass for thousands of metabolites, small peptides, and xenobiotic metabolites found across the plant and animal kingdom.

Because of this diversity in physical properties and size of the metabolome in biological systems, there has been a need for the development of several analytical protocols based on different chromatographic methods to select specific subgroups of metabolites based on these different chemical properties beyond the technical capabilities of LC-MS and GC-MS. These new methods have their own advantages for selecting specific types of molecules: lipids, nucleotides, aminoacids or steroids. Non-mass spectrometric methods such as nuclear magnetic resonance (NMR) and non-chromatographic methods such as matrix-assisted laser desorption- time of flight (MALDI-TOF) imaging are successfully being used for selected metabolomic analyses. The focus of this review is recent publications that use CE-MS to extend the polar metabolome beyond what is observed by LC-MS and GC-MS.

### Metabolomics – current methods

Using the appropriate technology can be a critical decision when starting a discovery program. Different methods or technical platforms have advantages within certain chemical spaces, and the choice of platform can effect linear dynamic range, lower level of quantitation,

resolution of isomers, baseline biological noise and in-source ion suppression. No universal methods exist so care should be made when making conclusions in evaluating unbiased and untargeted metabolomic data. With the diversity of metabolites captured by any one method, one can expect a range of different responses effecting dynamic range and linearity of response depending upon the specific metabolite properties. The choice of technology can affect the number of false positives, how to assess quality control and the type of statistical analysis in any metabolomics study.

The most commonly used separation method uses high-pressure liquid chromatography (HPLC). HPLC methods vary to accommodate a broader range of metabolites. Ion pairing, ion exchange, hydrophilic interaction chromatography (HILIC) and reverse phase columns can all be used to select specific subtypes of metabolites. Capillary electrophoresis (CE) offers a novel approach with distinct advantages as seen in a growing list of publications.

### CE mechanism

Understanding that HPLC and GC methods are not all inclusive, Smith et al. [1] demonstrated the potential of interfacing CE with mass spectrometry (MS) in 1987, although it was not until Soga et al. [2] published in 2002 that CE-MS could be performed with high reproducibility and sensitivity for biological applications. CE works using electrophoretic movement or electroosmotic flow (EOF) to govern transport and separation of metabolites.

EOF is a phenomenon where the electrolyte or running buffer solution itself flows inside the capillary. The flow of the electrolyte solution is the main driving force that pushes samples into the mass spectrometer side of the

capillary. A fused-silica capillary contains surface charges of silanol groups present on the inner walls. The silanol groups on the capillary inner wall are ionized presenting an overall negative surface. Opposing ions in the electrolyte solution are attracted to the inner wall surface to achieve a balance of electric charges, resulting in the formation of a double-layer with ionized silanol groups. Under these conditions, a potential difference is created very close to the inner wall. The application of a voltage to both ends of the capillary attracts the positively charged ions of the diffuse double-layer to an anode. In contrast, the silanol groups cannot move due to the fixation on the wall surface and the entire electrolyte solution in the capillary is directed toward the anode with the migration of the positively charged ions, thereby generating a flow.

The degree of mobility of any compound relative to others is due to variations in their ionic radius and size and charge of the electrolyte filling the capillary. A compound or metabolite with a larger ionic radius and smaller charge would have limited mobility compared to small, more polar species. Compounds or metabolites with a smaller ionic radius and higher charge would have high mobility. Hence controlling the electrical gradient across the capillary and pH of the electrolyte solution are two of the most important parameters in controlling metabolite separation into the mass spectrometer.

Hence CE-MS offers a totally different approach to metabolite separation prior to mass spectrometric detection. Earlier reviews go into more detail on how CE works, the theory of electroosmotic flow (EOF) and CE-MS interfaces [3,15,16,17,18].

### Early problems

Despite the strong need to broaden the ability to measure the hydrophilic metabolic space, the usage of CE-MS has been relatively slow. Sensitivity, mass spectrometric stability, migration time reproducibility, and correction software for migration time shift have all contributed to slow uptake. In addition, limited commercial CE-MS solutions and the lack of CE-MS availability in core academic, government or industrial laboratories has limited development in the field. Since CE-MS may measure many new molecules, not in LC libraries, the creation of new small molecule libraries and the peak picking and warping software needed for CE alignment has also slowed progress. Lastly, the ability to provide a stable, sensitive, and reliable interface has been a critical issue to overcome.

### Early developments

In 2002, Tomoyoshi Soga first developed a metabolome

analysis method based on CE-MS, which enabled the simultaneous analysis of several thousand charged metabolites by cationic and anionic methods [1,7,11,14]. Since then, CE-MS began to grow as one of the standard methods in bioscience research. See Soga's recent review in 2015 [18]. Soga and other labs have clearly demonstrated that most of these early issues and concerns have been overcome by technological improvements, hence the growing list of publications in this field since 2002. CE-MS protocols have been developed to support metabolic measurements, both relative and quantitative, in a large variety of sample types including urine [4,5,8], bacteria [6], CSF [9], neurons [10] and brain [12].

### Current advantages and biotechnology applications

Multiple comparative studies reviewed and discussed by Kok et al. [13,17] have shown that the metabolome fraction mapped by CE-MS is usually not covered by other techniques. Importantly, CE also gives complementary information to HILIC [13], as well as, significantly better peak shape in many cases. It is therefore not surprising that CE-MS has already been considered for multiple metabolomics studies covering several applications, from aging [19], amino acid analysis [20,21], bacteria [22-26], Cancer [27-43], CNS [46-53], CVD [54-58], diabetes [59-65], lifestyle [61-71], kidney disease [72,73], liver disease [74-82], the microbiome [83-89] and many other biological areas [90-108].

CE-MS is the most suited technique for analyzing phosphorylated metabolites, amino acids or metabolites from the TCA cycle and glycolytic pathways, all being key metabolites in multiple biochemical processes and gaining remarkable importance in metabolomics, including cancer research. Indeed, isomeric compounds such as citrate/isocitrate, as well as, leucine/isoleucine/also leucine, can be resolved and quantitated in biological matrices [153].

Another great advantage of CE-MS is its high quantification accuracy. CE-MS is less affected by LC-MS associated matrix effects, such as ion suppression and enhancement, due to the lack of mass transfer between solid and liquid phases in CE. CE uses uncoated fused silica capillary columns, the separation occurring by electrical interactions or EOF, unlike LC reverse phase and HILIC methods where peak broadening occurs by mass transfer between the liquid and solid phase. CE provides a flat solvent front compared to the convex front with HPLC separation systems. Peak spreading by CE is only due to longitudinal diffusion across the capillary column thus producing separations with better peak shape, greater dynamic range for certain metabolites over LC-MS meth-

**Table 1.** Reviews on capillary electrophoresis-mass spectrometry (year 2016)

| Authors   | Title   | Year | Ref.  |
|---|---|------|-------|
| Dao-Quan Tang, Li Zou, Xiao-Xing Yin, Choon Nam Ong       | HILIC-MS for metabolomics: An attractive and complementary approach to RPLC-MS          | 2016 | [109] |
| Klampfl CW.   | Capillary Electrophoresis-/Liquid Chromatography-Mass Spectrometry 2016                 | 2016 | [110] |
| Ramautar R.   | Chapter one: Capillary Electrophoresis-Mass Spectrometry for Clinical Metabolomics      | 2016 | [111] |
| Zhang W, Hankemeier T, Ramautar R.                        | Next-generation capillary electrophoresis-mass spectrometry approaches in metabolomics. | 2016 | [112] |
| Ramautar R.   | CE-MS in metabolomics: status quo and the way forward.                                  | 2016 | [113] |
| Kohler I, Giera M.  | Recent advances in liquid-phase separations for clinical metabolomics.                  | 2016 | [114] |
| Iadarola P, Fumagalli M, Bardoni AM, Salvini R, Viglio S. | Recent applications of CE- and HPLC-MS in the analysis of human fluids.                 | 2016 | [115] |
| Garcia A, Godzien J, Lopez-Gonzalez A and Barbas J.       | Capillary electrophoresis mass spectrometry as a tool for untargeted metabolomics       | 2017 | [116] |
| Maier TV, Schmitt-Kopplin P.                              | Capillary Electrophoresis in Metabolomics   | 2016 | [117] |

ods. CE has also been favourably compared to HILIC separations offering higher flexibility, lower complexity, better RT reproducibility, higher peak capacity and better peak shape [17]. Another challenge to the popularity of CE-MS has been the effect of high salt concentrations on migration time reproducibility. High salt concentrations in the sample can lead to stacking and peak spreading affecting metabolite resolution, identification and mass accuracy. However, this issue can be avoided by determination of an appropriate dilution ratio for general types of samples (blood, tissue, urine, saliva etc.). Alternatively, some sample types may require a pre-test utilizing a small amount of sample. Lastly, one could use some measurement, such as electric conductivity or creatinine concentration in urine, to determine appropriate dilution ratio. CE-MS systems are currently available from SCIEX CESI 8000 (Redwood City, CA USA) and Agilent 7100 CE (Santa Clara, CA USA) providing both instruments and software as a viable path for individual laboratories. A contract research organization (Human Metabolome Technologies –Tsuruoka, Japan) can also provide CE-MS services, both quantitative and relative measurements for any matrix with protocols for any type of metabolomics interest.

With many technological challenges overcome, the advantages of CE-MS are clearly understood. With the availability of commercial CE-MS instrumentation, development in academic labs and contract labs and with a

growing list of publications and reviews [1-108], we saw in 2016 a balance of papers reflecting the diversity and growth of CE-MS in biotechnology and drug development.

### Over 200 CEMS publications in 2016

In this paper recent CE-MS applications developed for metabolomics covering the literature from Jan 2016 to Dec 2016 are outlined in **Tables 1, 2 and 3**. Attention will be paid to CE-MS approaches for the profiling of metabolites in the fields of biomedical, clinical and microbial metabolomics.

### Reviews

Reviews provide a short cut to obtaining specific and pertinent information about a topic. They also give a time table context for developing technologies or research areas. Several reviews of CE-MS were published in 2016 summarizing the growth of this field. **Table 1** summaries 10 reviews in 2016 on CE-MS, each with a different focus or agenda. Issue 37 of the Journal Electrophoresis [110] provided an entire publication to LC-MS and CE-MS, demonstrating equal status, although the CE-MS applications were not specific to metabolomics. Hankemeier's laboratory at Leiden University provided 4 separate reviews [111-114] covering different aspects of CE-MS from growing applications to technical developments. Tang et al. [109] provides a discussion comparing HILIC

**Table 2.** Technical and methods papers on CE-MS based metabolomics (year 2016)

| Authors  | Title  | Keywords             | Ref.  |
|--|--|----------------------|-------|
| Hernández-Mesa M, Cruces-Blanco C, García-Campaña AM.                            | Capillary electrophoresis-tandem mass spectrometry combined with molecularly imprinted solid phase extraction as useful tool for the monitoring of 5-nitroimidazoles and their metabolites in urine samples. | SPE on-line          | [118] |
| Yamamoto M, Ly R, Gill B, Zhu Y, Moran-Mirabal J, Britz-McKibbin P.              | Robust and High-Throughput Method for Anionic Metabolite Profiling: Preventing Polyimide Aminolysis and Capillary Breakages under Alkaline Conditions in Capillary Electrophoresis-Mass Spectrometry.        | Anionic metabolites  | [119] |
| Boizard F, Brunchault V, Moulos P, et al.  | A capillary electrophoresis coupled to mass spectrometry pipeline for long term comparable assessment of the urinary metabolome.   | Beveled tip          | [120] |
| D'Orazio G, Hernández-Borges J, Asensio-Ramos M, Rodríguez-Delgado MÁ, Fanali S. | Capillary electrochromatography and nano-liquid chromatography coupled to nano-electrospray ionization interface for the separation and identification of estrogenic compounds.                              | Multiplatform        | [121] |
| Hiroyuku Yamamoto and Kazunori Sasaki  | Metabolomics-based approach for ranking the candidate structures of unidentified peaks in capillary electrophoresis time-of-flight mass spectrometry   | Peak identification  | [122] |
| Gulersonmez, M. C., Lock, S., Hankemeier, T., Ramautar, R.,                      | Sheathless capillary electrophoresis-mass spectrometry for anionic metabolic profiling.  | Sheathless interface | [123] |
| Pont, L., Benavente, F., Jaumot, J. et al.                                       | Metabolic profiling for the identification of Huntington biomarkers by on-line solidphase extraction capillary electrophoresis mass spectrometry combined with advanced data analysis tools.                 | SPE on-line          | [124] |
| González-Peña D, Dudzik D, Colina-Coca C et al.                                  | Multiplatform metabolomic fingerprinting as a tool for understanding hypercholesterolemia in Wistar rats.  | Multiplatform        | [125] |
| Mastrangelo A, Martos-Moreno GÁ, García A et al.                                 | Insulin resistance in prepubertal obese children correlates with sexdependent early onset metabolomic alterations.   | Multiplatform        | [126] |
| Mastrangelo A, Panadero MI, Pérez LM et al.                                      | New insight on obesity and adipose-derived stem cells using comprehensive metabolomics.  | Multiplatform        | [127] |

chromatography to capillary electrophoresis with advantages and challenges for both. In *Methods in Molecular Biology* (2016) a whole chapter is provided [117] on CE-MS in metabolomics with detailed protocols and technical information for those getting into this field.

### Technology and protocols

Innovative technologies continue to advance through the ingenuity of researchers, providing reliable improvements in the technologies and protocols. Such advancements are needed in order to provide solutions to increasingly difficult analytical problems seen in the medical and

pharmaceutical fields. Over the past year, several publications (**Table 2**) are noted demonstrating new ideas and concepts designed to expand and improve CE-MS. Untargeted profiling, when including multiple analytical methods, provides the greatest opportunity to discover biomarkers, understand biological pathways and identify new drug targets. The University of San Pablo published 3 papers in 2016 [125-127] using multiple mass spectrometry based untargeted technologies [LC-MS, CE-MS and GC-MS] to create large data rich metabolomics profiles in plasma [125], serum [126] and from cell culture [127] to identify biomarkers in obesity and hypercholes-

**Table 3.** Application Papers using CE-MS based Metabolomics (year 2016)

| Authors  | Title   | Sample Type           | Size of study   | Groups                    | Disease Area        | Ref.  |
|--|---|-----------------------|---|---------------------------|---------------------|-------|
| Harada S, Takebayashi T, Kurihara A et al.   | Metabolomic profiling reveals novel biomarkers of alcohol intake and alcohol-induced liver injury in community-dwelling men.  | Human Plasma          | 47ND, 46LD, 52MD, 48HD exploratory with 193 replication                                 | Non drinkers to Drinkers  | Alcohol intake      | [128] |
| Miyamoto T, Hirayama A, Sato Y et al.  | A serum metabolomics-based profile in low bone mineral density postmenopausal women.  | Human serum           | 42 high, 5 low  | high BMD vs low BMD       | Osteoporosis        | [129] |
| N Yoshimi, T Futamura, S E Bergen et al.   | Cerebrospinal fluid metabolomics identifies a key role of isocitrate dehydrogenase in bipolar disorder: evidence in support of mitochondrial dysfunction hypothesis | Human CSF             | 54, 40  | BP vs HC                  | Bipolar Disorder    | [130] |
| Gao P, Zhou C, Zhao L, Zhang G, Zhang Y.   | Tissue amino acid profile could be used to differentiate advanced adenoma from colorectal cancer.   | colon tissue          | set1: 11 va 11, set2: 22 vs 10  | CRC vs colorectal adenoma | CRC                 | [131] |
| Kazuhiko Uchiyama, Nobuaki Yagi, Katsura Mizushima et al.  | Serum metabolomics analysis for early detection of colorectal cancer  | Human Serum           | 56 patients with CRC (n = 14 each of Stages I-IV), 60 healthy controls, and 59 patients | CRC stages vs HC          | CRC                 | [132] |
| Saito T, Sugimoto M, Okumoto K et al.  | Serum metabolome profiles characterized by patients with hepatocellular carcinoma associated with hepatitis B and C.  | Human serum           | 30, 38  | HCC-B vs HCC-C            | HCC with HepB, HepC | [133] |
| Hidenari Hirata, Keishi Sugimachi, Hisateru Komatsu et al.   | Decreased Expression of Fructose-1,6-bisphosphatase Associates with Glucose Metabolism and Tumor Progression in Hepatocellular Carcinoma                            | HuH7 HepG2 cell lines | Wt vs over expression of FBP1 as new cancer target                                      |                           | HCC                 | [134] |
| Yusuke Ochiai, Yasuo Uchida, Sumio Ohtsuki, Masanori Tachikawa, Sanshiro Aizawa and Tetsuya Terasaki | The blood-brain barrier fatty acid transport protein 1 (FATP1/SLC27A1) supplies docosahexaenoic acid to the brain, and insulin facilitates transport                | HEK293 cells          | HEK293 FATP1 expressing and mock HEK293 cells   |                           | CNS disease         | [135] |

**Table 3.** Continued

|  |  |   |                       |  |   |       |
|--|--|---|-----------------------|--|---|-------|
| Noriko Yoshimura, b, Takashi Futamura, Keiji Kakumoto et al.           | Blood metabolomics analysis identifies abnormalities in the citric acid cycle, urea cycle, and amino acid metabolism in bipolar disorder | Human Serum                                     | 39C, 54BD             | BP vs HC   | Bipolar Disorder                                  | [136] |
| Kageyama Y, Kasahara T, Morishita H et al.                             | Search for plasma biomarkers in drug-free patients with bipolar disorder and schizophrenia using metabolome analysis.                    | Human plasma                                    | 6, 17, 9, 19          | Bipolar Dis., Schiz., MDD, HC                                  | Bipolar Disorder and Schizophrenia                | [137] |
| Fujii T, Hattori K, Miyakawa T, Ohashi Y, Sato H, Kunugi H.            | Metabolic profile alterations in the postmortem brains of patients with schizophrenia using capillary electrophoresis-mass spectrometry. | Human brain Frontal Cortex and Hippocampus      | 15 vs 15              | Disease and normal   | Schizophrenia                                     | [138] |
| Nozawa S, Sato T, Katayama K, Ishioka K, Sako T, Arai T, Tazaki H.     | Metabolic analysis of canine peripheral blood mononuclear cells treated ex vivo with dexamethasone.                                      | canine PB-MCs                                   | with and without drug | Effects of dexamethasone on glucose metabolism                 | IR, diabetes                                      | [139] |
| Barbas-Bernardos C, Armitage EG, García A et al.                       | Looking into aqueous humor through metabolomics spectacles – exploring its metabolic characteristics in relation to myopia.              | Human aqueous humor after cataract surgery      | 12 vs 24              | high myopia and low myopia                                     | myopia - eye disease                              | [140] |
| Hamuro J, Ueno M, Asada K, Toda M, Montoya M, Sotozono C, Kinoshita S. | Metabolic Plasticity in Cell State Homeostasis and Differentiation of Cultured Human Corneal Endothelial Cells                           | Cultured human corneal endothelial cells cHCECs |                       |  | Regenerative eye disease                          | [141] |
| Serrano-Villar S, Rojo D, Martínez-Martínez M et al.                   | Gut bacteria metabolism impacts immune recovery in HIV-infected individuals.   | Human gut and plasma                            | 8, 9, 12, 8           | HC non-HIV, naive, responders, non-responders (6 longitudinal) | HIV -antiviral therapy (ART)                      | [142] |
| Hiroki Yoshimatsu, Atsushi Yonezawa, Kaori Yamanishi et al.            | Disruption of Slc52a3 gene causes neonatal lethality with riboflavin deficiency in mice  | Mouse Liver                                     | 3, 3, 3               | WT, -/- KO, -/- KO with Vit. B2                                | Inborn Metabolic Disorder (Riboflavin metabolism) | [143] |

Table 3. Continued

|   |  |                                      |                                     |  |   |       |
|---|--|--------------------------------------|-------------------------------------|--|---|-------|
| Kimura T, Yasuda K, Yamamoto R, Soga T, Rakugi H, Hayashi T, Isaka Y.                                 | Identification of biomarkers for development of end-stage kidney disease in chronic kidney disease by metabolomic profiling. | Human plasma                         | 112 samples                         | Stages 3, 4, 5                           | CKD End Stage Hazard Model                | [144] |
| Ayako Aoki-Yoshida, Reiji Aoki, Naoko Moriya, Tatsuhiko Goto et al.                                   | Omics Studies of the Murine Intestinal Ecosystem Exposed to Subchronic and Mild Social Defeat Stress                         | Mouse feces, cecal                   | 5 vs 5                              | Control and stressed                     | Stress-induced gastric response           | [145] |
| Ishikawa S, Sugimoto M, Kirabatake K et al.   | Identification of salivary metabolomic biomarkers for oral cancer screening.   | Saliva                               | 24 vs 44                            | Oral cancer vs control                   | Oral cancer                               | [146] |
| Jumpei WASHIO, Tamaki OGAWA, Keisuke SUZUKI, Yosuke TSUKIBOSHI, Motohiro WATANABE, Nobuhiro TAKAHASHI | Amino acid composition and amino acid-metabolic network in supragingival plaque  | Dental plaque                        | 16 samples                          |  | Dental disease                            | [147] |
| Yuka Torii, Yoshihiko Kawano, Hajime Sato et al.  | Quantitative metabolome profiling reveals the involvement of the kynurenine pathway in influenza-associated encephalopathy   | Human Serum                          | 12E, 22NE                           | Influenza associated                     | Encephalopathy                            | [148] |
| Iida M, Harada S, Kurihara A et al.   | Profiling of plasma metabolites in postmenopausal women with metabolic syndrome.   | plasma, women                        | 594 exploratory, 283 replication    |  | Metabolic Syndrome (MetS)                 | [149] |
| Fukai K, Harada S, Iida M et al.  | Metabolic Profiling of Total Physical Activity and Sedentary Behavior in Community-Dwelling Men                              | Human plasma                         | 808 exploratory and 385 replication | 4 levels of activity                     | Physical activity                         | [150] |
| Chano T, Avnet S, Kusuzaki K, Bonucelli G, Sonveaux P, Rotili D, Mai A, Baldini N.                    | Tumour-specific metabolic adaptation to acidosis is coupled to epigenetic stability in osteosarcoma cells.                   | Human cell cultures                  | 20 samples                          | Osteosarcoma cells vs normal fibroblasts | Acidosis in osteosarcoma, HDAC inhibitors | [151] |
| Gao P, Yang C, Nesvick CL et al.  | Hypotaaurine evokes a malignant phenotype in glioma through aberrant hypoxic signaling.                                      | Glioblastoma Multiforme brain tissue | 32 (grades 1-4; 4,11,10,7) vs 18 HC | Cancer vs non-cancer                     | Glioma PHD2 as new cancer target          | [152] |

terolemia. D’Orazio et al. [121] compared nano LC and CE-MS to quantitatively measure estrogenic metabolites from mineral water. These nano techniques were used to minimize sample volumes and reagent costs. While the phenyl-LC method provided the better calibration curves, CE-MS, using only electro-osmotic flow (EOF), demonstrated the capability to resolve and measure 11 estrogens including two isobaric compounds.

One of the long-standing issues with CE-MS is the ease and stability of the CE to MS interface. Ramautar [123] and Boizard [120] published on their work to improve the reliability and sensitivity of the MS interface. Ramautar provided data on using a sheathless capillary interface to improve the sensitivity with profiling glioblastoma cell lines, while Boizard designed a beveled capillary tip to improve the stability of the CE-MS interface, examining one sample over 130 times in 4 years. Improvements in sample preparation prior to CE separation was also seen in 2016 with two papers [119,124] using solid phase extraction [SPE] to improve detection sensitivity. Pont et al. [124] utilized on-line SPE with CE-MS to identify biomarkers of Huntington’s disease from mouse plasma. [R6/1]. Yamamoto et al. [119] provide protocols to help stabilize the long-term stability of anionic CE-MS by developing fused silica friendly methods. Lastly, Yamamoto and Sasaki [122] provided multi-step processes to aid in the identification of unknowns in CE-MS using accurate mass and data base mining. Protocols to expand the known metabolome are critical to exploiting the full potential of CE-MS.

### Medical applications

While there were over 200 CE-MS based publications in 2016, this review has focused on those with metabolomics applications and methods. Those 25 publications related to drug development are summarized in **Table 3**. These publications cover many therapeutic and disease areas, different sample types and study sizes reflecting the diversity and growth in this area.

Oncology has a great unmet need for biomarkers and treatment protocols. Omic technologies, like metabolomics, provides pathways to understanding cancer and treatment effects in both pre-clinical and clinical settings. Colorectal cancer (CRC) is one area of active clinical research. Two publications [131,132] were looking at either serum or colon tissue from CRC patients to develop markers for early detection or a more accurate diagnosis using CE-MS. Hepatocellular carcinoma (HCC) is a primary malignancy of the liver and occurs predominantly in patients with underlying chronic liver disease and cirrhosis, now the third leading cause of cancer deaths

worldwide. Hirata et al. [134] and Soga et al. [133] used CE-MS in cells and serum to help understand HCC at the cellular level and discover potential biomarkers. Hirata’s work provides additional support for FBP1 as a therapeutic target, while Soga delivers a series of inflammatory peptides in serum that helps to differentiate Hepatitis B and Hepatitis C effects on HCC patients. Other publications look at diverse sample types; saliva [146], cell culture [151] and brain tissue [152] for biomarkers and pathway analysis of oral cancer, osteosarcoma and glioma. CE-MS provides accurate measurements of critical small molecules associated with aberrant cancer metabolism and inflammation, representing targets for cancer therapeutic development.

While cancer research represents a major investment in the medical community, diseases of the central nervous system (CNS) continue to grow. Bipolar disorder (BD), formerly called manic depression, causes extreme mood swings that include emotional highs (mania or hypomania) and lows (depression). Three groups published using 3 different sample types, plasma [137], CSF [130] and serum [136] to both understand the biology of BD and seek biomarkers for diagnosis. Hashimoto [130] aptly used the specificity of CE-MS to understand the role of mitochondrial dysfunction in BD, finding isocitrate as a diagnostic biomarker in CSF. Likewise, the same group looked at serum TCA cycle metabolites using CE-MS to resolve and identify specific energy metabolites in BD sera. While Fuji [138] used the uniqueness of CE-MS to identify metabolites in brain tissue specific to Schizophrenia.

The remainder of **Table 3** lists publications related to lifestyle; alcohol intake [125], physical activity [150], microbiome [142,145,147] and to various diseases; eye disease [140, 141], inborn errors of metabolism [143], kidney disease [144], diabetes [139], metabolic syndrome [149], osteoporosis [129] and encephalopathy [148]. These publications represent a variety of subjects and sample types [serum plasma, cultured cells, PBMCs, tissue] that are amenable to CE-MS metabolomics profiling, specifically, polar hydrophilic metabolites found in critical pathways such as amino acid metabolism, central energy metabolism, inflammation, stress oxidative pathways, glycolysis, cancer metabolism, and lipid transportation.

### Future

The successful implementation and integration of metabolomics in personalized medicine and drug development will rely on the combination of high-throughput analysis, large metabolite coverage, accurate quantitation, high-value data and low-cost analysis. This paradigm can

only be achieved using state-of-the-art analytical technologies and computational techniques combining many modalities and data input with growth in technology and implementation. CE-MS is poised to make significant contributions to these fields as the technology and availability to CE-MS continues to increase.

Multi-platform, multi-omics approaches with large studies will develop high-powered algorithms for diagnostic and prognostic studies. Increasing CE databases, improvements in sheathless interfaces and increases in MS detection capability will further push the field forward helping to find solutions to critical questions in drug development and biotechnology.

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