1 Introduction

In the field of metabolomics, advanced separation techniques coupled to MS are frequently used for the global profiling of (endogenous) low-molecular weight compounds in biological samples [1]. Among the various separation methods, CE is especially suited for the analysis of (highly) polar and charged compounds as the CE separation mechanism is based on charge-to-size ratio. The first application of CE–MS for the global profiling of charged metabolites was demonstrated for bacterial extracts, in which more than 1600 molecular features were detected [2].

In this study, the analysis of cationic metabolites was performed with a bare fused-silica capillary using 1 M formic acid (pH 1.8) as BGE, while the analysis of anionic metabolites was performed with a cationic polymer-coated capillary using 50 mM ammonium acetate (pH 8.5) as BGE [2, 3].

Until now, within the field of metabolomics, CE–MS utilizing a classical sheath-liquid interface has been primarily used for the analysis of cationic metabolites employing a bare fused-silica capillary and low-pH separation conditions [4–7]. Only a few studies reported the development of CE–MS methods for the analysis of anionic metabolites, and in each study, different separation conditions were employed [3, 8–11]. The CE–MS system most often used for anionic metabolic profiling was developed by Soga and coworkers in 2002 [3]. In this set-up, CE was coupled to MS via a sheath-liquid interface and a cationic polymer-coated capillary employing 50 mM ammonium acetate (pH 8.5) as BGE in combination with reversed CE polarity was utilized for the analysis of anionic metabolites. At a later stage, it was demonstrated by the same group that this method led to corrosion of the stainless steel ESI needle and repeated capillary clogging unless a platinum sprayer needle was used [12]. However, the platinum sprayer is not required when CE separations are performed under normal polarity conditions.

The issues observed with the CE–MS method most frequently applied to anionic metabolic profiling may have led to the perception that CE–MS is not a reliable analytical technology for metabolomics studies, as substantiated by a cross-platform comparison study by Bürcher et al. [13]. In this study, CE–MS was suggested as the least suitable analytical technology for metabolomics studies. The authors found that the approach based on using the cationic polymer-coated capillary for anionic metabolic profiling under alkaline conditions is not a reliable analytical technology for metabolomics studies.
conditions lacked the robustness required for analyzing biological samples. Moreover, in a recent work presented by Britz-Mckibbon and coworkers at the 31st International Symposium on MicroScale Bioseparations it was shown that alkaline ammonia-based buffers (pH > 9) react with polyimide outer coatings of fused-silica capillaries resulting in frequent capillary fractures and poor long-term performance. Apart from these aforementioned limitations, global metabolomics studies by CE-MS are generally performed by using two different approaches, i.e. cationic metabolic profiling on a bare-fused silica capillary employing low-pH separation conditions and anionic metabolic profiling on a coated capillary employing high-pH separation conditions. This can be considered a time-consuming procedure as it takes time to adjust one CE-MS system into another (e.g. capillary reconditioning and coating; test runs with QC samples to assess coating stability and performance; changing sheath-liquid composition and flow rate). When all these aspects are taken into consideration, there is a strong need for a reliable CE-MS method for anionic metabolic profiling, which is also suitable for cationic metabolic profiling using exactly the same capillary and separation conditions, thereby reducing analysis time and providing one single analytical platform for the global profiling of polar and charged metabolites.

Recently, the field of CE-MS has benefitted from advances in interfacing designs allowing highly sensitive analyses [14]. In this context, the sheathless porous tip interface, the flow-through microvial interface and the EOF driven sheath-liquid interface emerged as important developments [15–17]. Ramautar et al. demonstrated that a significant improved metabolic coverage can be obtained for various biological samples with sheathless CE-MS using a porous tip interface as compared to sheath-liquid CE-MS [18, 19]. So far, the sheathless CE-MS method has only been evaluated for cationic metabolic profiling studies (i.e. under positive ion mode conditions). Bonvin et al. recently found lower limits of detection for the determination of glucuronides in human urine using nonaqueous CE-MS employing a sheathless instead of a sheath-liquid interface in negative ionization mode [20].

In the present study, the performance of sheathless CE-MS using a porous tip sprayer was evaluated for the profiling of anionic metabolites in biological samples by using the same CE-MS platform as recently developed for cationic metabolic profiling [18]. A representative metabolite mixture was used to assess analytical parameters such as migration time and peak area repeatability, response linearity, and LODs. The applicability of the sheathless CE-MS method for anionic metabolic profiling was examined for glioblastoma cell line extracts.

2 Materials and methods

2.1 Chemicals

All chemicals used were of analytical grade or higher purity. A Millipore Q-guard water purifying system (Billerica, MA, USA) was used to obtain pure water. Methanol and isopropanol were purchased from Biosolve ( Valkenswaard, The Netherlands) and acetic acid from JT Baker (Philipsburg, NJ, USA). Sodium hydroxide and ammonium acetate were purchased from Fluka (Buchs, Switzerland).

2.2 Sheathless CE-MS

Sheathless CE-MS experiments were performed with a CESI 8000 instrument from ScieX (Brea, California, USA) employing an OptiMS CESI cartridge (30 µm id x 91 cm bare fused-silica capillary), which was thermostatted using recirculating liquid coolant regulated at 25°C, and coupled to a Sciex TripleTOF 5600+ MS system using the NanoSpray III source. ESI was performed in negative ionization mode by setting the ionspray voltage at 1.5 kV. The values for gas 1, gas 2, and temperature were 0, 0, and 50, respectively, and MS data were acquired in the m/z region from 65 to 1000. MS parameters were optimized employing an ESI tuning mix. For the electrophoretic separation, 10% acetic acid (pH 2.2) was used as BGE. After installation of an OptiMS CESI cartridge, the capillary was first flushed with methanol for 10 min at 50 psi, followed by water for 10 min at 50 psi and then with BGE for 10 min at 50 psi. Between sample injections, the capillary was flushed with water for 3 min at 50 psi, followed by 0.1 M sodium hydroxide for 3 min at 50 psi, then by water for 3 min at 50 psi and finally by the BGE for 4 min at 50 psi. The BGE used in the porous separation capillary was also used as buffer in the conductive liquid capillary. Between each run, the conductive liquid capillary was rinsed for 1 min at 50 psi with this BGE. All samples were injected hydrodynamically using a pressure of 2 psi for 60 s (corresponding to circa 20 nL, which is circa 3% of the capillary volume). When not in use, the capillary was filled with water and the capillary inlet and outlet were deposited in vials containing BGE and water, respectively, and stored at room temperature.

2.3 Test mixture and biological samples

A standard metabolite mixture comprised of 32 anionic compounds at a concentration of 50 µM in water was obtained from Human Metabolome Technologies (Tokyo, Japan). From this standard mixture, the following compounds, as they represent three different chemical families, i.e. organic acids, sugar phosphates, and nucleotides, were used for the evaluation of the performance of the sheathless CE-MS method: D-Ribose-5-phosphate, D-Glucose-1-phosphate, D-Glucose-6-phosphate, D-Fructose-6-phosphate, Citric acid, Isocitric acid, 2-Hydroxy-butyric acid, Trimesic acid, Inosine 5'-monophosphate (IMP), and Adenosine 3',5'-cyclic monophosphate (cAMP). Solutions of this test mixture were prepared in water at −80°C until usage. The intra- and interday repeatability of the sheathless CE-MS method for migration times and peak heights (or peak areas) were determined by ten repeated injections of the metabolite mixture (12.5 µM) on
three consecutive days. Linearity of response for the metabolite standards was evaluated by measurement of eight analyte concentrations ranging from 0.25 to 50 μM. LODs for the test compounds were calculated as the concentration yielding a S/N-ratio of 3 by further diluting the lowest concentration of the sample used for the construction of calibration curves (extracted ion electropherograms were used for this purpose) until the concentration yielded a S/N-ratio of 3. For anionic metabolic profiling, intracellular metabolites from the human U-87 MG glioblastoma cell line (89081402 Sigma-Aldrich), after cell lysis using ice-cold methanol/water (80/20, v/v) and ultrasonication, were extracted with a liquid–liquid extraction procedure using ice-cold water/methanol/chloroform (1/1/1, v/v/v). The methanol/water phase was evaporated and dried material was reconstituted in 50 μL water of which circa 20 nL (circa 3% of capillary volume) was injected into the sheathless CE-MS system corresponding to an amount of circa 400 cells.

2.4 Data analysis

CE-MS data were analyzed using PeakView software. Peak heights and areas of test compounds were determined from extracted ion electropherograms. The number of molecular features (i.e. the number of peaks detected above a certain intensity threshold within the CE run time) in a sample was determined manually using a S/N threshold of ≥ 5. In order to avoid accounting for the salts clusters observed in sheathless CE-MS for the analysis of biological samples, the number of molecular features in biological samples was only determined for the migration time region from 10 to 25 min. For the provisional identification of selected metabolites in a glioblastoma cell line extract, accurate mass information, and comparison of migration times with metabolite standards was used. A mass tolerance of 5 mDa was employed in order to (provisionally) identify the metabolites in the cell extracts.

3 Results and discussion

In the field of metabolomics CE-MS has been primarily used for the profiling of cationic metabolites until now. Concerning cationic metabolic profiling by CE-MS, there appears to be a consensus on the electrophoretic separation conditions to be employed for this purpose [4,7,21]. In general, a BGE of 1 M formic acid (pH 1.8) is used in the case of conventional CE-MS systems, i.e. employing a commercially available sheath-liquid interface, whereas a BGE of 10% acetic acid (pH 2.2) is often used in CE-MS systems based on one of the recently developed low flow interfaces techniques [18,22,23]. Though CE-MS has not frequently been used for anionic metabolic profiling, in part for reasons outlined in the Introduction, the various systems that have been reported on this topic all employ different separation conditions [3,10,24–26]. In this context, the aim of this study was to develop a CE-MS method for the highly efficient and sensitive profiling of anionic metabolites in biological samples, which could also be employed for the profiling of cationic metabolites without changing the capillary and separation conditions, thereby providing a generic CE-MS approach for metabolomics studies.

3.1 Evaluation of sheathless CE-MS for anionic metabolic profiling

Recently, a sheathless CE-MS method using a porous tip nanospray interface has been developed for the highly efficient, selective, and direct analysis of cationic metabolites in various body fluids using 10% acetic acid (pH 2.2) as BGE [18,19]. Nanomolar detection limits were obtained for a wide range of compound classes (i.e. amino acids, amines, nucleosides, and small peptides) and compared to sheath-liquid CE-MS systems this was an improvement of at least tenfold. In this study, the separation conditions from the previously developed sheathless CE-MS method were selected as a starting point for method development. In contrast to the previous study, a high-end QTOF-MS instrument was used in the present study. In order to allow the migration of negatively charged compounds to the MS at low-pH separation conditions, the CE instrument was operated in reverse polarity mode and a small pressure of 0.5 psi (~35 mbar) was applied at the inlet of the capillary in order to counteract the residual EOF present under these conditions. As reported by Soga et al. the use of reverse polarity under high pH BGE conditions in sheath-liquid CE-MS leads to corrosion of the stainless steel ESI needle and repeated capillary clogging unless a platinum sprayer needle is used [12]. In the sheathless porous tip interface, corrosion of the sprayer is not an issue as the emitter is a silica porous tip. The MS instrument was used in negative ion mode as most anionic metabolites are ionized more effectively under these conditions. Moreover, MS in negative ion mode is required for the detection of metabolites with only acidic groups.

The performance of the sheathless CE-MS method for anionic metabolic profiling was first assessed for the analysis of a set of compounds with different physicochemical properties and which belong to key metabolite classes, i.e. organic acids, sugar phosphates, and nucleotides. Figure 1 shows the analysis of the metabolite test mixture (25 μM) by sheathless CE-MS using an injection volume of circa 20 nL (corresponding to an amount of 500 fmol for each analyte). Under the employed separation conditions, most metabolites could be analyzed with a satisfactory detection sensitivity, adequate peak shapes, and with a good distribution along the migration time axis between 10 and 25 min. The migration time of a neutral marker (DMSO) was circa 25 min. signifying an effective separation window for anionic metabolites of 15 min if only the migration time region from 10 to 25 min is considered for the analysis of anionic metabolites. The sheathless CE-MS method was capable of resolving structurally similar metabolites, such as the isomers citric acid and isocitric acid and 2-hydroxybutyric acid and 3-hydroxybutyric acid (Fig. 1).
Figure 1. Multiple extracted ion electropherograms for the metabolite test mixture (25 μM) obtained with sheathless CE-MS in negative ion mode using a porous tip sprayer. Peaks: 1, 2-Naphtol-3,6-disulfonic acid; 2, D(+)-2-Phosphoglyceric acid; 3, D-Ribose-5-phosphate; 4, D-Glucose-1-phosphate; 5, D-Glucose-6-phosphate; 6, D-Fructose-6-phosphate; 7, Inosine 5'-monophosphate (IMP); 8, Guanosine 3',5'-cyclic monophosphate (cGMP); 9, Guanosine 5'-monophosphate; 10, Citric acid; 11, Trimesic acid; 12, Isocitric acid; 13, Gluconic acid; 14, Adenosine 3',5'-cyclic monophosphate (cAMP); 15, 2-Hydroxybutyric acid; 16, β-Diphosphopyridine nucleotide (NAD+); 17, 3-Hydroxybutyric acid. Experimental conditions: BGE, 10% acetic acid (pH 2.2); separation voltage, –30 kV (+0.5 psi applied at the inlet of the CE capillary); sample injection, 2.0 psi for 60 s.

Sugar phosphates and their structural isomers, could be partially separated by sheathless CE-MS under these conditions (Fig. 2), which is sufficient for their selective detection by MS. For the test compounds, plate numbers ranged from 60 000 to 400 000, indicating a high separation efficiency. On the basis of the effective separation window of 15 min and the average peak width (0.086 min) the peak capacity was circa 175 for this system. Overall, these results clearly illustrate the ability of the sheathless CE-MS method for the highly efficient and selective analysis of anionic metabolites, which often are difficult to analyze with conventional reversed-phase LC-MS methods.

Next, the repeatability of the method was examined by ten consecutive injections of the test mixture (12.5 μM of each compound). The intra- and interday variation of migration time and peak heights were determined for a
number of test compounds representing three chemical families, i.e. sugar phosphates, nucleotides, and organic acids. Table 1 shows that the intra- and interday RSDs for migration times and peak heights were below 2 and 11%, respectively, demonstrating an acceptable repeatability of the sheathless CE-MS method. These results are in line with repeatability data obtained previously for cationic metabolic profiling by sheathless CE-MS and also in agreement with other CE-MS-based metabolomics studies employing sheath-liquid interfaces [3, 9, 10, 18, 23, 27, 28].

The performance of the sheathless CE-MS method was further assessed by determining the linearity of response and LODs for the test compounds. Linear calibration curves were obtained in the range from 0.25 to 50 μM for all test compounds. LODs for the test compounds ranged between 10 and 200 nM when an injection volume of circa 20 nL was used (i.e. ~3% of the capillary volume), corresponding to 0.4–4 mol injected (Table 1), which is a significant improvement as compared to LODs reported in earlier CE-MS-based metabolomics studies. For example, Soga and coworkers obtained LODs (S/N = 3) between 1 and 7 μM for anionic metabolite standards by sheath-liquid CE-MS using an injection volume of 30 nL (i.e. mass detection limits ranged from 9 to 200 mol) [3]. LODs between 30 and 900 nM were obtained for anionic metabolites when the conventional sheath-liquid stainless steel sprayer was replaced by a platinum needle under the high-pH separation conditions in combination with a cationic coated capillary [12]. Using a similar approach as Soga and coworkers, but a different coated capillary, Timischl et al. found LODs around 1 μM for selected sugar phosphate, organic acid, and nucleotide standards [9]. Wakayama et al. recently developed a sheath-liquid CE-MS method for the simultaneous analysis of amino acids and small carboxylic acids using 1 M formic acid as BGE in normal CE polarity mode [29]. The analysis of carboxylic acids next to the amino acids at low-pH BGE conditions was only possible by using a sheath gas flow pressure of 25 psi, i.e. the liquid suction through the capillary caused by the sheath gas reduced the migration times of the carboxylic acids. By changing the detection polarity in ESI-MS from positive to negative ionization mode during the CE run, both amino acids and carboxylic acids could be analyzed. LODs for carboxylic acids ranged from 1 to 10 μM (e.g. 2.5 μM for isocitric and citric acid) that could be ascribed to the relatively high sheath-liquid flow-rate (10 μL/min), thereby significantly diluting the CE effluent. The performance of the method was not illustrated for sugar phosphates and nucleotides. We expect that the separation of structurally similar sugar phosphates is very challenging when using a sheath gas flow pressure of 25 psi.

In order to improve the concentration sensitivity of sheath-liquid CE-MS for anionic metabolic profiling, Kok et al. recently evaluated various BGE compositions and found the most optimal analyte responses with 25 mM triethylamine (pH 11.7), resulting in LODs (S/N = 3) between 0.7 and 9.1 μM when using an injection volume of circa 20 nL [10]. The method provided a LOD of 2.5 μM for citric acid and 1.5 μM for D-Glucose-6-phosphate, while the sheathless CE-MS method presented in this paper provided an LOD of 0.2 and 0.1 μM, respectively, for these analytes that is an at least tenfold improvement. Moreover, the use of triethylamine as BGE can be problematic as the ion pair reagent can contribute to major ion suppression or enhancement effects when using the same MS instrument under positive ion conditions.

Overall, sheathless CE-MS employing 10% acetic acid (pH 2.2) as BGE can be used for the highly efficient and sensitive analysis of anionic metabolites, such as sugar phosphates, organic acids, and nucleotides. The method is capable of separating structurally similar metabolites, which is of pivotal importance for their selective detection by MS. By only changing the polarity of the MS detection mode and the CE

| Compound                        | Intraday MT RSDs (%) | Intraday peak height RSDs (%) | Interday MT RSDs (%) | Interday peak height RSDs (%) | Detection limit (nM) at a S/N = 3 |
|---------------------------------|----------------------|-------------------------------|----------------------|-------------------------------|----------------------------------|
| D-Ribose-5-phosphate            | 0.9                  | 8.4                           | 1.1                  | 9.8                           | 100                              |
| D-Glucose-1-phosphate           | 0.9                  | 9.2                           | 1.1                  | 10.2                          | 50                               |
| D-Glucose-6-phosphate           | 0.9                  | 10.4                          | 1.2                  | 10.7                          | 100                              |
| D-Fructose-6-phosphate          | 0.9                  | 10.5                          | 1.2                  | 10.7                          | 100                              |
| Citric acid                     | 1.1                  | 8.0                           | 1.3                  | 9.7                           | 200                              |
| Isocitric acid                  | 1.1                  | 7.8                           | 1.3                  | 9.6                           | 40                               |
| 2-Hydroxybutyric acid           | 1.0                  | 9.7                           | 1.3                  | 10.2                          | 100                              |
| Trimesic acid                   | 0.9                  | 7.2                           | 1.2                  | 9.0                           | 50                               |
| IMP                             | 0.9                  | 8.4                           | 1.1                  | 9.5                           | 100                              |
| cAMP                            | 0.8                  | 9.5                           | 1.2                  | 10.5                          | 10                               |

Experimental conditions: BGE, 10% acetic acid (pH 2.2); separation voltage, –30 kV (+0.5 psi applied at the inlet of the CE capillary); sample injection, 2.0 psi for 60 s.
Figure 3. Multiple extracted ion electropherograms for a selected number of molecular features observed in an extract of a glioblastoma cell line with sheathless CE-MS in negative ion mode using a porous tip sprayer. Experimental conditions: BGE, 10% acetic acid (pH 2.2); separation voltage, –30 kV (+0.5 psi applied at the inlet of the CE capillary); sample injection, 2.0 psi for 60 s.

Figure 4. Multiple overlaid extracted ion electropherograms for two molecular features observed in an extract of a glioblastoma cell line with sheathless CE-MS in negative ion mode using a porous tip sprayer for three consecutive injections. Experimental conditions: BGE, 10% acetic acid (pH 2.2); separation voltage, –30 kV (+0.5 psi applied at the inlet of the CE capillary); sample injection, 2.0 psi for 60 s.

voltage, the sheathless CE-MS method can be directly used for the profiling of cationic metabolites, demonstrating the flexibility and potential of this approach for global metabolic profiling studies.

3.2 Application of the method to metabolic profiling of biological samples

In order to investigate the potential of the proposed sheathless CE-MS platform for the profiling of anionic metabolites in biological samples, extracts of the glioblastoma cell line were analyzed. Using manual inspection, the average number of molecular features (a feature is a detected signal at a given migration time and m/z ratio, presumably corresponding to a detected molecule) observed by the sheathless CE-MS method in the glioblastoma cell line extract above a S/N-ratio ≥ 5 was 122 (SD ≈ 6) for three repeated injections employing an injection volume of circa 20 nL, which is the equivalent of 400 cells per analysis. The 122 features include isotopic peaks of metabolites, so the actual number of metabolites detected is lower than this number. Figure 3 shows multiple overlaid selected ion electropherograms for a selected number of molecular features detected in the glioblastoma cell line extract, indicating that these features represent authentic analyte signals. Compounds like pyruvic acid, lactic acid,
citric acid, some sugar phosphates, and nucleotides could be clearly detected on the basis of accurate mass and the comparison of migration times with metabolite standards. At this stage, however, the aim of this study was not to characterize the metabolome of glioblastoma cell line extracts but to assess whether the profiling of anionic metabolites in this biological sample could be performed in a repeatable way with the sheathless CE-MS method. Therefore, RSDs for migration times and peak areas were determined for a few selected metabolites observed with different responses in the cell line extract by the sheathless CE-MS method. As an example, Fig. 4 shows multiple overlaid extracted ion electropherograms for two molecular features with an $m/z$ value of 89 0243 (Lactic acid) and 606 0743 (UDP N-acetylglucosamine) for three repeated injections of an extract of a glioblastoma cell line. The RSDs for migration times and peak areas of these analytes were below 0.7 and 7.5%, respectively, indicating that stable anionic metabolic profiles were obtained by this method.

The proposed sheathless CE-MS method can also be directly used for the profiling of cationic metabolites by simply changing CE in normal polarity mode and the MS in positive ionization mode, thereby keeping all the other parameters constant. A highly information-rich profile for cationic metabolites was observed for the analysis of circa 20 nL in a glioblastoma cell line extract, without employing any derivatization. An injection volume of circa 20 nL resulted in detection limits between 10 and 200 nM for test compounds, which corresponded to a significant improvement as compared to LOD values obtained with sheath-liquid CE-MS systems. A nice feature of the proposed methodology is the ability to also profile cationic metabolites by merely changing the CE polarity and the MS detection mode. Therefore, by just employing two sample injections a global profile for anionic and cationic metabolites can be obtained for a given biological sample. At this stage, the current analytical platform is only suitable for the screening of metabolites in relatively small sets of biological samples. On the basis of the results obtained with three porous tip capillaries, a single porous tip capillary can be used on average for the analysis of 92 (SD = 19) samples. The next step is to assess the long-term performance of this method for anionic metabolic profiling of large sets of biological/clinical samples. For quantitative studies aspects like matrix effects and accuracy need to be evaluated as well. The multisegment injection CE-MS strategy.

4 Concluding remarks

In this study, sheathless CE-MS using a porous tip sprayer has been evaluated for the analysis of anionic metabolites. A favorable migration time and peak area repeatability were obtained for test compounds and for some selected endogenous metabolites in a glioblastoma cell line extract, without employing any derivatization. An injection volume of circa 20 nL resulted in detection limits between 10 and 200 nM for test compounds, which corresponded to a significant improvement as compared to LOD values obtained with sheath-liquid CE-MS systems. A nice feature of the proposed methodology is the ability to also profile cationic metabolites by merely changing the CE polarity and the MS detection mode. Therefore, by just employing two sample injections a global profile for anionic and cationic metabolites can be obtained for a given biological sample. At this stage, the current analytical platform is only suitable for the screening of metabolites in relatively small sets of biological samples. On the basis of the results obtained with three porous tip capillaries, a single porous tip capillary can be used on average for the analysis of 92 (SD = 19) samples. The next step is to assess the long-term performance of this method for anionic metabolic profiling of large sets of biological/clinical samples. For quantitative studies aspects like matrix effects and accuracy need to be evaluated as well. The multisegment injection CE-MS strategy.
first described by Staub et al. for pharmaceutical studies [30], and recently evaluated by the group of Britz-McKibbin for high-throughput metabolomics may be very useful to evaluate for this purpose [31, 32]. Another important aspect to tackle is the identification of metabolites, which in general is an issue in global metabolomics studies. Therefore, our next step is to construct a CE-MS-based compound library for a large set of anionic and cationic metabolite standards that is based on recorded electrophoretic mobilities, accurate mass, and product ion scans. Such data would highly facilitate the (provisional) identification of (some) metabolites in biological samples. In order to further improve the concentration sensitivity of the sheathless CE-MS method for anionic metabolic profiling various electrophoretic preconcentration techniques, such as, for example transient-isotachophoresis, will also be evaluated in the future. As a final point, the proposed sheathless CE-MS methodology for anionic metabolic profiling need to be compared with ion-pair reversed-phase LC-MS, ion-exchange LC-MS, and HILIC-MS approaches in order to determine the actual complementary character of this technique for the profiling of anionic compounds.

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Dr. Stephen Lock has a conflict of interest as he is affiliated with Sciex.

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5 References

[1] Kuehnbaum, N. L., Britz-McKibbin, P., Chem. Rev. 2013, 113, 2437–2468.
[2] Soga, T., Ohashi, Y., Ueno, Y., Naraoka, H., Tomita, M., Nishioka, T., J. Proteome Res. 2003, 2, 488–494.
[3] Soga, T., Ueno, Y., Naraoka, H., Ohashi, Y., Tomita, M., Nishioka, T., Anal. Chem. 2002, 74, 2233–2239.
[4] Hirayama, A., Wakayama, M., Soga, T., Trac-Trend Anal. Chem. 2014, 61, 215–222.
[5] Ramautar, R., Somsen, G. W., de Jong, G. J., Electrophoresis 2009, 30, 276–291.
[6] Ramautar, R., Somsen, G. W., de Jong, G. J., Electrophoresis 2015, 36, 212–224.
[7] Britz-McKibbin, P., Methods Mol. Biol. 2011, 708, 229–246.
[8] Soga, T., Ishikawa, T., Igarashi, S., Sugawara, K., Kakazu, Y., Tomita, M., J. Chromatogr. A 2007, 1159, 125–133.
[9] Timischl, B., Dettmer, K., Kaspar, H., Thieme, M., Oefner, P. J., Electrophoresis 2008, 29, 2203–2214.
[10] Kok, M. G., de Jong, G. J., Somsen, G. W., Electrophoresis 2011, 32, 3016–3024.
[11] Harada, K., Ohaya, Y., Tabushi, T., Kobayashi, A., Fukusaki, E., J. Biosci. Bioeng. 2008, 105, 249–260.
[12] Soga, T., Igarashi, K., Ito, C., Mizobuchi, K., Zimmermann, H. P., Tomita, M., Anal. Chem. 2009, 81, 6165–6174.
[13] Buscher, J. M., Czernik, D., Ewald, J. C., Sauer, U., Zamboni, N., Anal. Chem. 2009, 81, 2135–2143.
[14] Lindenburg, P., Haselberg, R., Rozing, G., Ramautar, R., Chromatographia 2015, 78, 367–377.
[15] Moini, M., Anal. Chem. 2007, 79, 4241–4246.
[16] Maxwell, E. J., Zhong, X., Zhang, H., van Zeijl, N., Chen, D. D., Electrophoresis 2010, 31, 1130–1137.
[17] Wojcik, R., Dada, O. O., Sadilek, M., Dovič, N. J., Rapid Commun. Mass Spectrom. 2010, 24, 2554–2560.
[18] Ramautar, R., Busnel, J. M., Deelder, A. M., Mayboroda, O. A., Anal. Chem. 2012, 84, 885–892.
[19] Ramautar, R., Shylt, R., Schoenmaker, B., de Groot, L., Derks, R. J., Ferrari, M. D., van den Maagdenberg, A. M., Deelder, A. M., Mayboroda, O. A., Anal. Bioanal. Chem. 2012, 404, 2895–2900.
[20] Bonvin, G., Schappler, J., Rudaz, S., J. Chromatogr. A 2014, 1323, 163–173.
[21] Ramautar, R., Somsen, G. W., de Jong, G. J., Metabolomics in Practice, Wiley-VCH Verlag, Weinheim, 2013, pp. 177–208.
[22] Lindenburg, P. W., Ramautar, R., Jayo, R. G., Chen, D. D., Hankemeier, T., Electrophoresis 2013, 35, 1308–1314.
[23] Hirayama, A., Tomita, M., Soga, T., Analyst 2012, 137, 5026–5033.
[24] Liu, J. X., Aerts, J. T., Rubakhin, S. S., Zhang, X. X., Sweedler, J. V., Analyst 2014, 139, 5835–5842.
[25] Hui, J. P., Yang, J., Thorson, J. S., Soo, E. C., Chembiochem 2007, 8, 1180–1188.
[26] Kami, K., Fujimori, T., Sato, H., Sato, M., Yamamoto, H., Ohashi, Y., Sugiyama, N., Ishihama, Y., Onozuka, H., Ochiai, A., Esumi, H., Soga, T., Tomita, M., Metabolomics 2013, 9, 444–453.
[27] Chalcraft, K. R., Britz-McKibbin, P., Anal. Chem. 2009, 81, 307–314.
[28] Ramautar, R., Torano, J. S., Somsen, G. W., de Jong, G. J., Electrophoresis 2010, 31, 2319–2327.
[29] Wakayama, M., Aoki, N., Sasaki, H., Oh sugi, R., Anal. Chem. 2010, 82, 9967–9976.
[30] Staub, A., Rudaz, S., Veuthy, J. L., Schappler, J., J. Chromatogr. A 2010, 1217, 8041–8047.
[31] Kuehnbaum, N. L., Kormendi, A., Britz-McKibbin, P., Anal. Chem. 2013, 85, 10664–10669.
[32] Kuehnbaum, N. L., Gillen, J. B., Kormendi, A., Lam, K. P., DiBattista, A., Gibala, M. J., Britz-McKibbin, P., Electrophoresis 2015, doi:101002/elps.201400604.