Published in final edited form as:

Anal Chim Acta. 2017 July 25; 978: 55–60. doi:10.1016/j.aca.2017.04.044.

Alternate Injections Coupled with Flow-Gated Capillary Electrophoresis for Rapid and Accurate Quantitative Analysis of Urine Samples

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Abstract

Capillary electrophoresis (CE) is a powerful separation technique with advantages over HPLC in terms of separation efficiency, speed, and cost. However, CE suffers in poor reproducibility in quantitative chemical analysis, which is one of major drawbacks preventing its widespread use in routine analytical laboratories. Here we report a novel strategy to enhance the quantitative capability of flow-gated CE. The platform integrated dual flow branches to respectively supply a sample and its standard additions that were then alternately injected into a single capillary for rapid separations (typically 20–90 s). A micro-fabricated switch was used to enable the alternate injections. It was assumed that the analytical system maintained constant conditions during neighboring injections that served as external self-standards for quantitation. This strategy was expected to reduce uncertainties caused by the fluctuation in capillary conditions and the drift of detection systems. Experimental results demonstrated that the dual-branch flow-gated CE coupled with alternate injections significantly improved the reproducibility with respect to peak height ratios under deliberate variations in injection volumes, separation voltages, optical focusing, and laser power; and thus the interday precision was ensured. To demonstrate its applicability, cyanide and amino acids in human urine were quantified rapidly with the one-point standard addition method after fluorogenic derivatization with naphthalene-2,3-dicarboxaldehyde (NDA), and the measurement accuracy was validated by determining the recovery of standard cyanide added to a urinary matrix. This strategy would be valuable to enable the quantitative capability of flow-gated CE in the measurements of a broad range of analytes, especially those lacking suited internal standards.

Graphical abstract

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Keywords

Alternate injections; Standard addition method; Capillary electrophoresis; Cyanide detection; Amino acid analysis

1. Introduction

Capillary electrophoresis (CE) is a powerful separation technique over HPLC in terms of separation efficiency, analytical speed, and operational cost [1–3]. Since its renaissance attributed to Jorgenson and Lukacs in the early 1980s [4,5], CE-related instrumentation and technologies have been considerably advanced [6]. However, the real-world application of CE is still limited in routine analytical laboratories. This unpopularity is mainly due to two major drawbacks: detection sensitivity and reproducibility [7]. The detection sensitivity has been significantly improved by using sensitive detectors such as laser-induced fluorescence (LIF) and mass spectrometry (MS) [8–10]; and nano- or pico-molar limits of detection (LODs) have been achieved via appropriate detection configuration and/or various sample preconcentration techniques [11–13]. However, the reproducibility of CE is still a concern although many efforts have been made to overcome this barrier [14]. This obstinateness is mainly attributed to the inconsistent injection and unstable migration of analytes [15].

Typically, hydrodynamic or electrokinetic injection is employed to introduce a sample plug into a separation capillary. To compensate for the potential variation of injection volumes or amounts, an internal standard (IS) should be selected and used in quantitative analysis with CE [16]. The IS method works better in the hydrodynamic injection than the electrokinetic due to its associated injection bias of analytes with different mobilities. In addition, an appropriate IS is often hard to find for specific analytes; a single IS may not be simultaneously suited for multiple analytes; and the LIF detection has higher requirements for the IS due to the non-uniform photo-bleaching effect on different fluorophores [17]. In the electrokinetic injection, it is essential to maintain a stable electroosmotic flow (EOF) to ensure reproducible injections. However, almost always, the EOF fails to be stable on an inter-day basis due to various factors including sample matrices, background electrolytes (BGE pH, composition, and concentration), temperature, and non-specific adsorption on capillary walls [15]. As a routine procedure, frequent capillary re-conditioning is performed in conventional CE to refresh inner capillary walls, but this procedure cannot ensure the capillary recovery. Therefore, how to enhance the quantitative reproducibility of CE becomes a challenging but crucial task for further research and development.

For quantitative analysis of complex samples, the standard addition method (SAM) is preferred to the standard calibration curve since the SAM can compensate for rotational

matrix effects [18]. The multiple-point SAM was usually considered superior to the one-point SAM with regard to the measurement accuracy. However, the multiple-point SAM assumes that the zero response corresponds to a zero concentration of an analyte, but it often fails to be true [18]. Moreover, the response linearity in the concentration range of standard additions should be verified, but it becomes impractical and time-consuming due to the complexity of matrices [18,19]. Alternatively, the one-point SAM has been advocated and should be seriously considered [18,19]. Theoretical and experimental results have established that the one-point SAM can be precise and accurate in quantitation when appropriate experimental conditions are used [19,20]. Specifically in CE, sample matrices affect sample injection and analytes' migration besides conventional matrix effects, which may cause inaccuracy of measurements when using the multiple SAM. In addition, the multiple-point SAM lacks analytical throughput since multiple points at least three replicates each are required to quantify a single sample. On the other hand, the one-point SAM reduces the number of standard additions thus facilitating the analytical throughput. Moreover, flow-gated CE using a short capillary and a high separation voltage [21–24] or microchip CE is able to perform rapid separations which further improve the analytical throughput.

Flow-gated CE mimics the configuration of a single-cross microchip CE in which the flow-gated injection is frequently used for effective flow control and sample injection [25,26]. Compared with microchip CE, flow-gated CE has numerous advantages. First, fresh BGE is supplied via a syringe, which reduces the pH variation due to electrolysis. Second, a silica capillary with various inside diameters may produce better separation efficiency thanks to its feasible surface property and the circular channels. Third, a silica capillary is more compatible with various detectors for sensitive detection. It is convenient to couple with a MS detector, and to use a sheath flow cuvette or an oil-immersion objective for LIF. Fourth, the separation capillary, if getting clogged or broken, can be easily replaced with a new one at a low cost. Especially when a polydimethylsiloxane (PDMS) flow gate is used, assembling a new capillary to the flow gate becomes more convenient [25,27].

In this paper, we report a novel strategy for rapid quantitation of biological samples by using the one-point SAM on a flow-gated CE system coupled with alternate injections of a sample and its standard additions. A PDMS micro-switch was fabricated and integrated with the flow-gated CE system to enable alternate injections. This method assumed that the detection system was stable during two neighboring separations in a short time. As a proof of concept, cyanide and amino acids in human urine were determined on the platform by using the one-point SAM.

2. Experimental

2.1 Chemicals and Reagents

Sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$), phosphoryethanolamine (PEA), taurine, and all amino acids were purchased from Sigma (St. Louis, MO). Potassium cyanide, dimethylformamide (DMF), sodium dodecyl sulfate (SDS) and tetrasodium salt of ethylenediaminetetraacetic acid (EDTA) were purchased from Fisher Scientific (Chicago, IL). Naphthalene-2,3-dicarboxaldehyde (NDA) was from Invitrogen (Eugene, OR). Hydroxypropyl-beta-cyclodextrin (HP- β -CD) was purchased from ACROS (New Jersey,

USA). Fused silica capillaries were ordered from Polymicro Technologies (Phoenix, AZ). Silicon wafers at 3 inches in diameter were ordered from University Wafer, Inc. (South Boston, MA).

The NDA stock solution at 10.0 mM was prepared in DMF, and the working NDA was prepared by diluting the stock solution to 2.0 mM or 5.0 mM with deionized (DI) water on a daily basis. The stock borate buffer (pH 9.2) was prepared by dissolving tetraborate (100.0 mM) in DI water. The stock solution of PEA (400.0 mM) was prepared in 100 mM tetraborate, and the working PEA was diluted to 80.0 mM unless otherwise stated. EDTA at 100.0 mM was prepared in DI water without pH adjustment. Cyanide derivatization was performed by off-line mixing of NDA (2.0 mM in DMF/water at 50/50 by volume), PEA (80 mM in 80 mM tetraborate pH 9.2 plus 10 mM EDTA), and cyanide sample at 1:1:1 volume ratio. Dual sample flows were supplied via micro-syringes powered by a Hamilton syringe pump at a flow rate of 600 nL/min.

2.2 Fabrication of micro-switch

The design of the micro-switch is shown in Fig. 1c which was fabricated with PDMS by following the reported procedure with appropriate modification [28]. Briefly, the master mold of the fluidic layer was prepared on the surface of a silicon wafer by using standard photolithography. First, SU-8 photoresist features at 60 μm in thickness and in width were fabricated on a silicon wafer to accommodate capillaries at 150 μm in OD and 40 μm in ID for external connections. Second, AZ photoresist features at 20 μm in thickness and 200 μm in width were prepared by aligning with SU-8 features to generate the valving channels. Finally, the AZ layer was rounded at 120 $^{\circ}\text{C}$ on a hot plate for 5 minutes. The mold for the control layer at 60 μm in thickness and 300 μm in width was prepared by using SU-8, and the bottom cover layer was prepared with a clean silicon wafer. The fluidic membrane (~60 μm in thickness) was spin-coated with a PDMS prepolymer mixture at 25:1 (mass/mass) of RTV615A and RTV615B purchased from Momentive Performance Materials (Waterford, NY), and other layers used 10:1 mixture. PDMS layers were cured at 80 $^{\circ}\text{C}$ in an oven for > 30 minutes. The three layers were permanently attached together after surface activation with a hand-held corona discharge treater (Electro-Technic Products, Chicago, IL). Tygon tubing for the nitrogen pressure was connected to the control channels through stainless steel tubes inserted into PDMS access holes punched with a blunted needle [29].

2.3 Instrumental setup and experimental conditions

As shown in Fig. 1a, the instrumental setup has been described previously [13,25]. Briefly, the excitation wavelength was 442 nm, and the emission was 485 ± 12.5 nm. The microscope used a 40x oil-immersion objective with the numerical aperture of 1.3 (Carl Zeiss Microscopy, Thornwood, NY, USA). The photomultiplier tube (PMT, Part # R11540) was ordered from Hamamatsu Photonics (Japan), and a current preamplifier (SR570) was purchased from Stanford Research Systems (Sunnyvale, CA, USA). A 4-syringe pump (Chemyx Inc., Stafford, TX, USA) was used to supply a sample and its standard additions through individual gastight Hamilton syringes (Reno, Nevada, USA). The flow gate and other interfaces were fabricated with poly(dimethylsiloxane) (PDMS) as described earlier [25]. The two-branch schematic diagram is shown in Fig. 1a. Connectors and the flow gate

were prepared with PDMS. The separation capillary had 10 μm in ID, 10 cm in effective length, and 17 cm in total. The separation voltage was 25 kV unless otherwise stated.

Phosphoryethanolamine (PEA) or cyanide, and naphthalene-2,3-dicarboxaldehyde (NDA) were used to fluorogenically derivatize cyanide or amino acids, respectively, for LIF detection. Urine samples were donated by volunteers, and were two-fold diluted by using NaOH at 20 mM. The BGE for cyanide separation was tetraborate (40 mM) at pH 9.2, and for amino acids separation was 40 mM tetraborate, 50 mM sodium dodecyl sulfate (SDS), and 1.0 mM hydroxypropyl-beta-cyclodextrin (HP- β -CD). (Refer to Supporting Information for more details about experimental conditions).

3. Results and discussion

3.1 Micro-switch Performance

Fig. 1b shows an image of the micro-switch assembly with control lines. Two solenoid valves were respectively connected to Controls I and II (see Fig. 1c). Note that Control II was connected to the same pressure line. Each branch of incoming sample flows had 2-way options: to the injection flow gate or to the waste, selected by two 3-way micro-switches. The micro-switch was manipulated by applying a nitrogen pressure (typically 5–10 psi) to close an opposite pair of valving channels while simultaneously opening the other pair. The open-close cycles were activated by using two solenoid valves controlled by a LabVIEW program which had three options: open Branch 1, open Branch 2, and alternate Branches 1 and 2. The timing of the alternate open-close cycle was set at the immediate completion of the sample injection, and thus the separation was being performed while the other sample flow was rinsing the common sample channel interconnecting the switch to the flow gate, and consequently next injection would be the second sample. This process repeated until being terminated.

Flow-gated CE was able to perform rapid separations typically in 20–90 s for the capillary at 10 μm in ID and 10 cm in effective length [22]. During the separation period, the dead volume contained in the switch and the common capillary between the switch and the flow gate should be completely replaced by the second sample. The micro-switch allowed a short common capillary (3–5 cm in length and 40 μm in ID), which significantly reduced the total dead volume. It is important to address that the dead volume (e.g. blue dye in Fig. 1c) between Control II (depressed in Fig. 1c) and the common capillary might leak and mix with the red dye; however, leaking was not observed due to the pushback by the red dye flow, which was attributed to the flexibility of PDMS fluidic channels. It took about 6 s to rinse the common capillary when the flow rate was set at 1600 nL/min, and 20 s when at 600 nL/min. The flow rate should be high enough to completely rinse the common capillary prior to the completion of a run, and it was safe to set up a higher flow rate than necessary to ensure cross-contamination free. Fig. 1d shows the comparison of peak heights observed in alternate injections or individuals, which indicates that the cross contamination between the two samples was negligible when the common channel was being flushed at the flow rate of 600 nL/min for 40 s.

3.2 Reproducibility of measurements

The robustness of an analytical technique or instrument can be demonstrated by using the precision of measurements, which is presented as reproducibility or repeatability. Reproducibility refers to the measurement precision under potential sources of variability including “different locations, operators, measuring systems, conditions, and times”, and repeatability is defined as the precision of measurements in a short period of time on the same instrument, location, and operator [30]. To evaluate the capability of alternate injections in the flow-gate CE system, experiments were performed with deliberate variations in multiple technical parameters including injection volumes, separation voltages, laser power, and optical focusing. These parameters, as expected, significantly affected peak heights of cyanide derivatives as seen in Figs. S5–S8 and Table 1. However, the peak height ratios of neighboring separations maintained a constant value of 0.51, which was the expected ratio between 500 nM and 1000 nM plus the blank of cyanide in each derivatization mixture. The repeatability of CE measurements, namely run-to-run or intraday reproducibility, was usually satisfactory (see Table 1 and Fig. S3). However, the day-to-day or interday reproducibility as seen in Table 1 and Fig. S4 failed to be ideal despite that all the experimental parameters were maintained to the greatest extent. As the comparison, the migration time had the %RSD of 1.4 and 4.1 for intraday and interday experiments, respectively. This variation was attributed to the inherent complexity of CE, including injections, capillary conditions, and detectors. However, the ratio of alternate injections was preserved for both intraday and interday measurements. These results indicate that alternate injections in flow-gated CE were able to ensure the reproducibility even under extreme variations. It should be addressed that the number of neighboring pairs was equal to $n-1$ where n refers to the total number of alternate injections. Any outliers of these ratios indicate unexpected variation occurring during the involved injections.

3.3 Accuracy of measurements

The multiple-point SAM is usually considered more reliable than the one-point SAM in quantitative chemical analysis; however, extrapolation based on a standard addition calibration curve often generates inaccurate results [18]. In CE quantitation, specifically, a sample matrix has additional effects besides conventional matrix effects, which may include sample injections, analyte migration, and/or capillary conditions. These factors pose uncertainty to the calibration curve constructed by using a series of standard additions in a wide timespan. On the contrary, rapid separations in flow-gated CE coupled with alternate injections for the one-point SAM significantly decrease the experimental timespan that reduces chances of instrumental drift. Therefore, the one-point SAM has high potential to achieve accurate measurements. To validate the accuracy, four concentrations of standard cyanide were prepared in a urinary matrix, and the total concentration of each spiking was determined by using alternate injections and the one-point SAM. Similarly, the cyanide concentration in the urine matrix and the blank was also determined. Note that 1000 nM was used as the cyanide standard addition for all measurements, and the linearity up to 1500 nM was tested with the calibration curve of $y = 0.0024x + 0.0884$ (peak height against cyanide concentration in nM) and its correlation coefficient of $R^2 = 0.9989$. Four recoveries were calculated to be $(99 \pm 6)\%$, $(99 \pm 5)\%$, $(104 \pm 5)\%$, and $(97 \pm 5)\%$ for the spiking of 40, 100, 200, and 300 μM cyanide, respectively. These results indicate that the one-point SAM based

on alternate injections was able to obtain accurate quantitation. It should be addressed that the measurement accuracy heavily depends on the accuracy of standard additions. In addition, it is essential to maintain the same flow rates in the two sample branches thus ensuring repeatable injections; therefore, channel clogging or liquid leaking should be avoided.

3.4 Measurements of cyanide in urine

Cyanide quantitation in biological fluids is desired for forensic science and for testing cyanide exposure [31]. Available methods involve complex sample preparation procedures which may cause the loss of cyanide. Recently, Zhang et al. reported a rapid and direct method for the determination of cyanides in urine by using flow-gated CE and the multiple-point SAM [32], which required a series of standard additions to construct a calibration curve. Unfortunately, there were no appropriate internal standards for cyanide measurement using the method based on the fluorogenic derivatization with NDA in the presence of glycine. Here, the one-point SAM for the measurement of cyanide in urine was demonstrated by using alternate injections. PEA was selected as the amine for the fluorogenic derivatization of cyanide considering derivatives of NDA and PEA had better solubility than glycine or taurine (Fig. S1). Precipitation formation has been observed and was presumably attributed to the byproducts of NDA and amino acids [33]. Numerous primary amines exist in urine samples, and these amines compete for cyanide during the derivatization process. Therefore, a high concentration of PEA was experimentally selected to be 80 mM before off-line mixing (see Fig. S2).

Subsequently, cyanides in urine samples from smokers and non-smokers were determined by using the one-point SAM coupled with alternate injections. Typical electropherograms of cyanide and its standard addition were shown in Fig. 2. As can be seen, several small peaks appeared before and after the major peak, and they were attributed to derivatization by-products or derivatives of competitive amines in urine. Three urine samples from non-smokers had cyanide concentrations at 72 ± 4 , 128 ± 5 , 139 ± 8 nM, respectively; and the concentration range was 113 ± 36 nM. Three samples from smokers contained 237 ± 11 , 359 ± 8 , and 433 ± 15 nM, respectively, with the range of 335 ± 98 nM. Excluding the variation of biological samples, these results were considered reliable as confirmed by the accuracy validation. Apparently, cyanide levels in smokers' urine samples were significantly higher than those in non-smokers', and both concentration ranges agreed with reported results [34,35]. Note that off-line mixing was performed with NDA (in DMF/H₂O, 50/50), PEA, and the sample at a 1:1:1 ratio by volume, and the final mixture contained urine of 1/6, DI water of 4/6, DMF of 1/6 the total volume which produced an equivalent blank cyanide concentration of 24 nM in urine of 1/6 the total volume.

3.5 Amino acid analysis

Amino acid analysis determines the amounts of amino acids existing in biological fluids including blood plasma and urine, which aids in the identification of the balance of essential amino acids, the adequacy of dietary proteins, and/or the abnormal metabolism of proteins and amino acids [36,37]. Amino acid analysis also provides diagnostic information for various diseases. As a typical application of the one-point SAM coupled with alternate

injections for rapid and accurate measurements, 19 amino acids in urine were determined by using MEKC. Each urine sample was diluted by 10-fold with 20 mM NaOH and/or standard solutions to avoid fluorescence saturation in LIF detection. Considering level differences, standard additions were conducted by using three levels for three groups of amino acids: 15 μM of PEA, Glu, Asp, Val, Met, Ile, Trp, Leu, Phe and Arg, 50 μM of Ser, Asn, Thr, Gln, Ala, Tyr and Tau, and 100 μM of His and Gly. Fig. 3 shows typical neighboring electropherograms of a urine sample and its standard additions, and high separation efficiency was obtained with the number of theoretical plates $>300\text{ K}$ for each of the identified peaks. The limits of detection for these amino acids were estimated to be below 5.0 nM (at 3S/N) that agrees with the results using a similar detection system [38]. In addition, the fast separation of the 19 amino acids was completed in less than 2 min. As a comparison, liquid chromatography or conventional capillary electrophoresis for amino acid analysis usually takes a much longer time (e.g. 20–50 min) for a single run [39–41]. The level of each amino acid was determined by using the one-point SAM, and the results summarized in Table 2 agreed well with the reported ranges in urine samples [42]. It was also observed that Gly and His were the two most abundant amino acids, and Met and Ile were the least abundant out of the 19 amino acids identified. It should be pointed out that the dilution factor could be decreased to 2–5 folds when measuring the amino acids at low levels such as Met and Ile to ensure the accuracy and precision of measurements.

4. Conclusions

We have developed a novel strategy of alternate injections for flow-gated CE aiming to enable its quantitative capability. External self-standards via alternate injections in rapid electrophoretic separations provide a promising solution to the reproducibility problem in the quantitation of analytes lacking appropriate internal standards. In addition, the complexity of biological matrix imposes additional effects to quantitative analysis with CE besides the conventional matrix effect; therefore, the one-point SAM may be superior to the multiple-point SAM which may fail to generate accurate measurements due to the variability of CE separations.

Experimental validation has proven that the reproducibility of the ratio between alternate injections was maintained under deliberate variations of multiple factors, and the accuracy was confirmed by measuring the recovery of cyanide in a urinary matrix. To demonstrate its feasibility, this platform and the alternate injection strategy have been successfully applied to the rapid and accurate measurements of cyanide and amino acids in urine. The cyanide measurement based on fluorogenic derivatization of NDA and PEA is a typical example that lacks internal standards, and Amino acid analysis of urine samples is a typical example that lacks a single internal standard suited for simultaneous quantitation of multiple analytes.

Alternate injections of a sample and its standard additions would be a promising strategy to enable the quantitative capability of CE in a capillary or on a microchip. The high uncertainty of sample injection, analyte migration, and detection impose challenges to the broadly-defined precision, i.e. reproducibility. More complicated, to improve the detection sensitivity, online sample preconcentration via sample stacking, sweeping, or pH junctions introduces additional variables that significantly affect the reproducibility of measurements,

although these techniques may produce repeatable results in the narrowly-defined precision, i.e. repeatability. Moreover, microchip electrophoresis is able to perform rapid separations with high separation efficiency, but the reproducibility concern is even more intense than that in conventional CE. This alternate injection strategy is expected to be adapted to microchip electrophoresis in quantitative analysis thus improving the chip-to-chip reproducibility on various chip substrates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Thanks to Naveen Maddukuri for help with urine sample collection. This research was financially supported by the National Institute of General Medical Sciences with the grant number of P20 GM103418.

Abbreviations

BGE	background electrolyte
EOF	electroosmotic flow
HP-β-CD	hydroxypropyl-beta-cyclodextrin
IS	internal standard
LIF	laser-induced fluorescence
NDA	naphthalene-2,3-dicarboxaldehyde
PDMS	polydimethylsiloxane
PEA	Phosphoryethanolamine
SAM	standard addition method
SDS	sodium dodecyl sulfate

References

1. Xu Y. Tutorial: Capillary Electrophoresis. *The Chemical Educator*. 1996; 1:1–14.
2. Lian DS, Zhao SJ. Capillary Electrophoresis Based on Nucleic Acid Detection for Diagnosing Human Infectious Disease. *Clin Chem Lab Med*. 2016; 54:707–738. [PubMed: 26352354]
3. Pagaduan JV, Sahore V, TWoolley A. Applications of Microfluidics and Microchip Electrophoresis for Potential Clinical Biomarker Analysis. *Anal Bioanal Chem*. 2015; 407:6911–6922. [PubMed: 25855148]
4. Jorgenson JW, Lukacs KD. Capillary Zone Electrophoresis. *Science*. 1983; 222:266–272. [PubMed: 6623076]
5. Jorgenson JW, Lukacs KD. Zone Electrophoresis in Open-Tubular Glass Capillaries. *Anal Chem*. 1981; 53:1298–1302.
6. Geiger M, Hogerton AL, Bowser MT. Capillary Electrophoresis. *Anal Chem*. 2011; 84:577–596. [PubMed: 22148626]

7. Lerma-García MJ, Zougagh M, Ríos A. The Applied Side of Capillary Electrophoresis: A Critical View. *Curr Anal Chem*. 2014; 10:184–196.
8. Chen DY, Dovichi NJ. Yoctomole Detection Limit by Laser-Induced Fluorescence in Capillary Electrophoresis. *J Chromatogr B-Biomed Appl*. 1994; 657:265–269. [PubMed: 7952090]
9. Wojcik R, Dada OO, Sadilek M, Dovichi NJ. Simplified Capillary Electrophoresis Nanospray Sheath-Flow Interface for High Efficiency and Sensitive Peptide Analysis, *Rapid Commun. Mass Spectrom*. 2010; 24:2554–2560.
10. Klepárník K. Recent Advances in the Combination of Capillary Electrophoresis with Mass Spectrometry: From Element to Single-Cell Analysis, *Electrophoresis* 34 (. 2013:70–85.
11. Breadmore MC, Tubaon RM, Shallan AI, Phung SC, Abdul Keyon AS, Gstoettenmayr D, Prapatpong P, Alhusban AA, Ranjbar L, See HH, Dawod M, Quirino JP. Recent Advances in Enhancing the Sensitivity of Electrophoresis and Electrochromatography in Capillaries and Microchips (2012–2014). *Electrophoresis*. 2015; 36:36–61. [PubMed: 25330057]
12. Whitmore CD, Essaka D, Dovichi NJ. Six Orders of Magnitude Dynamic Range in Capillary Electrophoresis with Ultrasensitive Laser-Induced Fluorescence Detection, *Talanta* 80 (. 2009:744–748.
13. Zhang Q, Gong M. On-Line Preconcentration of Fluorescent Derivatives of Catecholamines in Cerebrospinal Fluid Using Flow-Gated Capillary Electrophoresis. *J Chromatogr A*. 2016; 1450:112–120. [PubMed: 27156734]
14. Breadmore MC. Capillary and Microchip Electrophoresis: Challenging the Common Conceptions. *J Chromatogr A*. 2012; 1221:42–55. [PubMed: 22000781]
15. Schaeper JP, Sepaniak MJ. Parameters Affecting Reproducibility in Capillary Electrophoresis. *Electrophoresis*. 2000; 21:1421–1429. [PubMed: 10826690]
16. Altria KD. Improved Performance in Capillary Electrophoresis Using Internal Standards, *LC-GC Europe* 15 (. 2002:588–594.
17. Wang GR. Laser Induced Fluorescence Photobleaching Anemometer for Microfluidic Devices. *Lab Chip*. 2005; 5:450–456. [PubMed: 15791344]
18. Ellison SLR, Thompson M. Standard Additions: Myth and Reality. *Analyst*. 2008; 133:992–997. [PubMed: 18645637]
19. Franke JP, De Zeeuw RA, Hakkert R. Evaluation and Optimization of the Standard Addition Method for Absorption Spectrometry and Anodic Stripping Voltammetry. *Anal Chem*. 1978; 50:1374–1380.
20. Brown RJC, Gillam TPS. Comparison of Quantification Strategies for One-Point Standard Addition Calibration: The Heteroscedastic Case. *Measurement*. 2012; 45:1670–1673.
21. Matysik FM. Advances in Fast Electrophoretic Separations Based on Short Capillaries. *Anal Bioanal Chem*. 2010; 397:961–965. [PubMed: 20306181]
22. Grundmann M, Matysik FM. Fast Capillary Electrophoresis-Time-of-Flight Mass Spectrometry Using Capillaries with Inner Diameters Ranging from 75 to 5 μ m. *Anal Bioanal Chem*. 2011; 400:269–278. [PubMed: 21311872]
23. Opekar F, Coufal P, Štulík K. Rapid Capillary Zone Electrophoresis Along Short Separation Pathways and Its Use in Some Hyphenated Systems: A Critical Review. *Chem Rev*. 2009; 109:4487–4499. [PubMed: 19537776]
24. Koenka IJ, Sáiz J, Rempel P, Hauser PC. Microfluidic Breadboard Approach to Capillary Electrophoresis. *Anal Chem*. 2016; 88:3761–3767. [PubMed: 26926522]
25. Zhang Q, Gong M. Prototyping of Poly(Dimethylsiloxane) Interfaces for Flow Gating, Reagent Mixing, and Tubing Connection in Capillary Electrophoresis. *J Chromatogr A*. 2014; 1324:231–237. [PubMed: 24331370]
26. Zhang X, Li Q, Chen Z, Li H, Xu K, Zhang L, Tang B. Electrokinetic Gated Injection-Based Microfluidic System for Quantitative Analysis of Hydrogen Peroxide in Individual Hepg2 Cells. *Lab Chip*. 2011; 11:1144–1150. [PubMed: 21298131]
27. Hooker TF, Jorgenson JW. A Transparent Flow Gating Interface for the Coupling of Microcolumn Lc with Cze in a Comprehensive Two-Dimensional System. *Anal Chem*. 1997; 69:4134–4142.
28. Unger MA, Chou HP, Thorsen T, Scherer A, Quake SR. Monolithic Microfabricated Valves and Pumps by Multilayer Soft Lithography. *Science*. 2000; 288:113–116. [PubMed: 10753110]

29. Cellar NA, Kennedy RT. A Capillary-Pdms Hybrid Chip for Separations-Based Sensing of Neurotransmitters in Vivo, Lab Chip 6 (. 2006:1205–1212.
30. Wegscheider W. Standardization, Quality-Control and Education in Analytical-Chemistry. Fresenius J Anal Chem. 1994; 349:784–793.
31. Lindsay AE, Greenbaum AR, O'Hare D. Analytical Techniques for Cyanide in Blood and Published Blood Cyanide Concentrations from Healthy Subjects and Fire Victims. Anal Chim Acta. 2004; 511:185–195.
32. Zhang Q, Maddukuri N, Gong M. A Direct and Rapid Method to Determine Cyanide in Urine by Capillary Electrophoresis. J Chromatogr A. 2015; 1414:158–162. [PubMed: 26342870]
33. Jackson R, Oda RP, Bhandari RK, Mahon SB, Brenner M, Rockwood GA, Logue BA. Development of a Fluorescence-Based Sensor for Rapid Diagnosis of Cyanide Exposure. Anal Chem. 2014; 86:1845–1852. [PubMed: 24383576]
34. Sano A, Takezawa M, Takitani S. High-Performance Liquid-Chromatography Determination of Cyanide in Urine by Pre-Column Fluorescence Derivatization. Biomed Chromatogr. 1989; 3:209–212. [PubMed: 2804427]
35. Jermak S, Pranaityte B, Padarauskas A. Headspace Single-Drop Microextraction with in-Drop Derivatization and Capillary Electrophoretic Determination for Free Cyanide Analysis, Electrophoresis 27 (. 2006:4538–4544.
36. urawicz E, Kafu na-Czapli ska J. Analysis of Amino Acids in Autism Spectrum Disorders, TrAC. Trends Anal Chem. 2015; 73:91–118.
37. Rutherford SM. Amino Acid Analysis of Plant Products. Amino Acids in Higher Plants. 2015:481–496.
38. Shou M, Smith AD, Shackman JG, Peris J, Kennedy RT. In Vivo Monitoring of Amino Acids by Microdialysis Sampling with on-Line Derivatization by Naphthalene-2,3-Dicarboxyaldehyde and Rapid Micellar Electrokinetic Capillary Chromatography. J Neurosci Methods. 2004; 138:189–197. [PubMed: 15325127]
39. Sharma G, Attri SV, Behra B, Bhisikar S, Kumar P, Tageja M, Sharda S, Singhi P, Singhi S. Analysis of 26 Amino Acids in Human Plasma by Hplc Using Aqc as Derivatizing Agent and Its Application in Metabolic Laboratory. Amino Acids. 2014; 46:1253–1263. [PubMed: 24515597]
40. Fekkes D. State-of-the-Art of High-Performance Liquid Chromatographic Analysis of Amino Acids in Physiological Samples. J Chromatogr B-Biomed Appl. 1996; 682:3–22. [PubMed: 8832421]
41. Lorenzo MP, Villaseñor A, Ramamoorthy A, Garcia A. Optimization and Validation of a Capillary Electrophoresis Laser-Induced Fluorescence Method for Amino Acids Determination in Human Plasma: Application to Bipolar Disorder Study. Electrophoresis. 2013; 34:1701–1709. [PubMed: 23512402]
42. Tan IK, Gajra B. Plasma and Urine Amino Acid Profiles in a Healthy Adult Population of Singapore. Ann Acad Med Singap. 2006; 35:468–475. [PubMed: 16902722]

Highlights

- Alternate injections for flow-gated capillary electrophoresis were enabled by using a microfabricated switch
- The one-point standard addition method was validated for rapid quantitation of biological samples with flow-gated CE
- Quantitative reproducibility was enhanced by using alternate injections coupled with rapid electrophoretic separations

Fig. 1.

(a) Interconnected flow-gated CE system. (b) Image of the micro-switch assembly. (c) Image of valving and control channels. Control II was depressed and Control I was released. Arrows indicate flow directions. (d) Peak height comparison between alternate injections and individuals. Branches 1 and 2 supplied 1.0 and 0.5 μM glycine derivatized with NDA, respectively. GND = Ground, LIF = Laser-induced fluorescence, and HV = High-voltage power supply.

Fig. 2.

Comparison of typical neighboring electropherograms with alternate injections of cyanide and its standard addition in urine. The separation capillary had 10 μm in ID, 10 cm in effective length, and 17 cm in total. The separation voltage was -25 kV. Injection was performed at -5 kV for 0.5 s. Separation buffer was tetraborate at 40 mM and pH 9.2 NDA at 2.0 mM in 50/50 DMF/water by volume, and PEA at 80 mM in 80 mM tetraborate pH 9.2. Off-line mixing and derivatization at 1:1:1 of NDA:PEA:Sample by volume.

Fig. 3.

Comparison of typical neighboring electropherograms with alternate injections of amino acids and their standard additions in urine. The separation capillary had 10 μm in ID, 10 cm in effective length, and 17 cm in total. The separation voltage was -22 kV. Injection was performed at -5 kV for 0.3 s. Separation buffer composed of tetraborate at 40 mM and pH 9.2, SDS at 50 mM, and HP- β -CD at 1.0 mM. 1 Ser, 2 Asn, 3 Thr, 4 Gln, 5 His, 6 PEA, 7 Gly, 8 Glu, 9 Ala, 10 Asp, 11 Tyr, 12 Tau, 13 Val, 14 Met, 15 Ile, 16 Trp, 17 Leu, 18 Phe, and 19 Arg. The insert indicates the zoom-in portion.

Table 1

Reproducibility validation.

CN ⁻ (μ M)	Average Peak Height (%RSD) ^a		
	0.50	1.00	Ratio ^f
Intraday	0.86 (3.4)	1.70 (3.3)	0.505 (1.3)
Interday	0.93 (8.5)	1.83 (8.2)	0.504 (1.2)
Voltage ^b	0.90 (28.7)	1.76 (29.2)	0.509 (1.4)
Injection ^c	0.73 (41.8)	1.43 (41.5)	0.508 (1.6)
Laser ^d	0.72 (12.5)	1.43 (13.1)	0.504 (1.7)
Focus ^e	0.59 (25.4)	1.16 (25.8)	0.513 (1.5)

^a n = 8 for each condition.^b 20, 25 and 30 kV.^c 0.5, 1.0 and 2.0 s at 5 kV.^d 10, 15 and 20 mW.^e Random for each pair of injections.^f Of neighboring pairs.

Table 2

Measured levels of amino acids in urine samples.

	Mean $\pm$ SD (μ M) ^a			
	Sample 1	Sample 2	Sample 3	Inter-samples
Ser	287 $\pm$ 12	263 $\pm$ 12	597 $\pm$ 20	382 $\pm$ 186
Asn	161 $\pm$ 10	95 $\pm$ 5	243 $\pm$ 12	166 $\pm$ 74
Thr	122 $\pm$ 5	137 $\pm$ 7	279 $\pm$ 7	179 $\pm$ 87
Gln	412 $\pm$ 19	498 $\pm$ 21	614 $\pm$ 28	508 $\pm$ 101
His	1077 $\pm$ 40	1092 $\pm$ 29	1677 $\pm$ 59	1282 $\pm$ 342
PEA	57 $\pm$ 2	72 $\pm$ 3	49 $\pm$ 2	59 $\pm$ 12
Gly	2171 $\pm$ 83	1221 $\pm$ 54	1988 $\pm$ 61	1793 $\pm$ 504
Glu	18 $\pm$ 1	38 $\pm$ 2	34 $\pm$ 1	30 $\pm$ 11
Ala	245 $\pm$ 10	318 $\pm$ 14	765 $\pm$ 32	443 $\pm$ 281
Asp	25 $\pm$ 1	23 $\pm$ 1	52 $\pm$ 2	33 $\pm$ 16
Tyr	67 $\pm$ 3	123 $\pm$ 6	147 $\pm$ 6	112 $\pm$ 41
Tau	988 $\pm$ 39	357 $\pm$ 19	409 $\pm$ 16	585 $\pm$ 350
Val	49 $\pm$ 2	82 $\pm$ 3	95 $\pm$ 5	75 $\pm$ 24
Met	6.5 $\pm$ 0.3	7.9 $\pm$ 0.4	11 $\pm$ 1	9 $\pm$ 2
Ile	12 $\pm$ 1	14 $\pm$ 1	22 $\pm$ 1	16 $\pm$ 5
Trp	44 $\pm$ 2	61 $\pm$ 2	72 $\pm$ 3	59 $\pm$ 14
Leu	23 $\pm$ 1	41 $\pm$ 2	39 $\pm$ 2	34 $\pm$ 10
Phe	42 $\pm$ 2	67 $\pm$ 3	66 $\pm$ 3	58 $\pm$ 14
Arg	60 $\pm$ 3	76 $\pm$ 2	53 $\pm$ 2	63 $\pm$ 12

^a%RSD < 5 based on n = 6 pairs of neighboring injections.