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Coupling of a large-size capillary column with an electrospray mass spectrometer
A reliable and sensitive sheath flow capillary electrophoresis–mass spectrometry interface

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Abstract

The concept of interfacing a large-size column for capillary electrophoresis (CE) to electrospray ionization mass spectrometry (ESI-MS) for robust and automatic CE–MS operation is reported. Both standard ionspray interface and microionspray interface have been modified to operate in a sheath flow pattern to overcome the common stability problem in CE–MS coupling. To make the interface sensitive, a step-down stainless steel tube with smaller inner diameter and tapered tip was incorporated onto a larger tube embracing the CE column via cold soldering. The devices were evaluated for quantitative analysis of nucleotides at femtomole level and stable analytical performance in peptide profiling.
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1. Introduction

The coupling of capillary electrophoresis (CE) with mass spectrometry (MS) has reached a significant level of maturity since the first publication on CE–MS in 1987 utilizing an electrospray ionization (ESI) interface [1]. In that report, a metal coating on the CE column end made contact with a metal capillary to which the electrospray voltage was applied. The following year, the same group presented another paper using the metal capillary as the sheath liquid transporter. The electrical contact for the CE circuit and the ESI emitter was maintained by the sheath liquid [2]. Also in 1988, a liquid junction interface was reported to couple CE with a pneumatically assisted electrospray (also called ion spray) MS [3]. In 1989, CE–MS employing continuous-flow fast-atom bombardment sources was reported employing either a liquid junction [4] or a co-axial sheath [5] interface.

ESI is the most versatile ionization technique in existence today [6]. The only absolute prerequisite for ESI is that the analyte of interest be soluble in a solvent, therefore, ESI affords a nearly ideal marriage between CE and MS. It will likely remain the preferred approach for the foreseeable future of CE–MS coupling. Although the acceptance of CE–MS has increased tremendously in the past decade, some limitations of this technique hinder its widespread application. For instance, on-column detection using UV absorption is straightforward; the detection end of the capillary can simply be placed in a buffer

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reservoir containing an electrode. For MS detection, however, an electrical connection of another kind has to be developed. A robust interface is crucial for stable and sensitive CE–MS operation and is still a hot topic. Other issues are related to the optimization of CE separations for real-sample applications of various kinds, specifically the use of buffers, and the choice of a mass analyzer for fast and efficient ion sampling and data acquisition. Ideally, the interface should maintain the separation efficiency and resolution of CE, be sensitive, precise and linear in response, maintain electrical continuity with the separation capillary, cope with all eluents present in CE, and provide efficient ionization from low flow-rates for mass analysis. Until recently, coupling of capillary zone electrophoresis (CZE) with MS has still been relying on coaxial sheath, liquid-junction and sheathless interfaces that were developed a decade ago.

Most sheathless interfaces utilize a metalized emitter made of fused-silica and a layer of metal coating such as silver [1] or gold [7–9]. The metal coating maintains the electrical contacts for CE and ESI operation, and the tapered tip increases the electric field for electrospray [10]. Other approaches are more complicated and delicate, for example inserting a metal electrode inside the outlet of the CE capillary [11,12]. The sheathless interface is generally the simplest for coupling CE to MS, leading to high sensitivity that is due to the low flow-rate and the high electrospray efficiency [13–17]. These interfaces are actually microspray or nanospray interfaces. The major reported drawback of these sheathless designs is the short lifetime mainly due to particle clogging and deterioration of the metal coating by electrical discharge, although improved coating methods have been developed to increase metallized emitter lifetime [18–21]. Another limitation of the sheathless interface for CE–MS analysis is that gas bubbles formed or transported to the tip can often terminate the electrospray and/or CE process. Furthermore, the choice of CE buffers is also restricted to a very narrow range due to the lack of post-column solution chemistry; the ESI response is fully dependent upon the CE buffers that are often substantially conductive and thus generate unstable electrospray. Since the tip is part of the separation capillary, modification to the tip (e.g. coating and tapering) requires special attention, especially for inner-coated capillaries. In general, the sensitivity gain is often compromised by poor signal stability [18].

In the liquid junction designs, a liquid gap is introduced between the CE column and the sprayer [22]. Impaired sensitivity and degraded separation efficiency has been reported because of the introduction of a gap in the system [23]. A conductively coated piece of fused-silica capillary butted to the CE separation capillary, as a disposable emitter has also been used [24–26].

A sheath flow interface normally utilizes a coaxial configuration. To date it has been the most commonly employed method for CE–MS coupling. In this arrangement, the CE column is inserted into a narrow metal tube that delivers a sheath liquid to the column exit. As the liquid flows through the tube, it mixes with the column effluent and completes the circuits for CE and electrospray. A sheath liquid, such as methanol, is chosen for its excellent electrospray characteristics and to overcome the volatility and conductivity problems of a CE buffer that usually has poor electrospray capability. The sheath liquid is supplied at a higher flow-rate than the electroosmotic flow (EOF); the ESI process is mainly dominated by the sheath liquid. The result is that a wide range of CE buffer compositions and solvents can be effectively selected, though with some constraints owing to background signals from the sheath liquid. Another advantage is that with the help of the sheath flow, gas bubbles generated by the electrolytic action of electrospray are not detrimental to the separation process. The main problem with this interface is the dilution of analyte caused by the sheath. Also, in some applications, the small magnitude of EOF may affect the electrical contact. When the EOF is minimal, a hydrodynamic flow through the CE capillary introduced by either raising the injection end for a few centimetres or applying a small constant pressure may aid electrical contact while incurring minimal degradation of the CE separation. Effects of sheath liquid composition on CE resolution and migration variations have been reported [27,28]. A silica sheath interface for low flow-rate electrospray CE–MS has also been introduced [29]. The sprayer tip is made of a fused-silica capillary or a borosilicate glass tube with a large

internal diameter (I.D.) and outer diameter (O.D.) pulled and tapered to a small opening. A similar approach with a CE column coated with gold at the detection end to improve the spray stability has been reported [30]. The use of these fused-silica interfaces for CE–MS applications, although reported to be successful, do not always result in a stable spray over a long period of time in our experience, mainly due to clogging and damaging of the tip.

Nowadays most commercially available ESI sprayers/sheath tubes are made of stainless steels with small dimensions to maintain ESI-MS sensitivity; therefore CE columns with 185 μm or less outer diameter have been chosen for CE–MS operations. CE manufacturers, however, often recommend the use of larger size columns, e.g. with 365 μm O.D., for ruggedness and reliability, especially with on-column UV detection. In this case, to interface the CE to MS creates a new challenge for commercially available ESI-MS platforms. The dimensions of the sheath tube need to be increased, while the sprayer opening needs to be maintained at minimal size for high sensitivity. We describe in this paper an approach to coupling any size of CE column with ESI-MS, and present results generated on triple quadrupole and quadrupole time-of-flight (Q-q-TOF) mass spectrometers.

2. Experimental

2.1. Materials

Fused-silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA). HPLC-grade methanol was purchased from J.T. Baker (Phillipsburg, NJ, USA). Ammonium acetate was obtained from Anachemia (Montreal, Canada). Model compounds, angiotensin I, angiotensin II, guanosine (G), guanosine monophosphate (GMP), guanosine diphosphate (GDP), and guanosine triphosphate (GTP) were from Sigma (St. Louis, MO, USA). Stock solutions of nucleotides were prepared in methanol–water (50:50). Samples were diluted in background electrolyte for tuning purposes, and spiked in horse plasma extracts for quantitative analysis. Peptides were dissolved in water and diluted with running buffer for further analysis.

2.2. Instrumentation

A QSTAR quadrupole time-of-flight mass spectrometer equipped with a modified IonSpray source (Applied Biosystems/MDS Sciex, Concord, Canada) coupled with a P/ACE MDQ automated CE system (Beckman Coulter, Fullerton, CA, USA) was employed to conduct CE–MS analysis of peptides. Fig. 1a shows the design of the sheath flow ionspray interface. The interface consists of a stainless steel microvolume tee. The sample line, usually the CE capillary column with outer diameter of up to 360 μm, is inserted into the electrode and extends past the through-bore of the first tee, and stops at the step-down of the stainless steel sprayer. The column is then pulled back 0.5–1 mm (see insert in Fig. 1). The injection end and the sprayer end of the capillary column were positioned at the same height to avoid siphoning. The sheath liquid supplied with a syringe pump (Harvard Apparatus, South Natick, MA, USA) comes in contact with the tee and the sprayer, and is charged under high voltages. The charged solution mixes with the column eluent and sprays through the step-down sprayer. The sheath liquid completes the circuits for CE and ESI process. Nebulizer gas is supplied and computer controlled. The use of nebulizing gas allows the interface to be operated at liquid flow-rates of up to 10 μl/min. The distance between the electrode and the curtain plate was 5–10 mm; the spray was off-axis. The curtain plate was operated in a normal way, i.e. 1 kV and normal curtain gas flow setting. A UV detection window on the column was opened at 10 cm from the injection end. Positive mode ESI-MS was operated with protonated ion signals recorded. Major MS parameters, e.g. the declustering potential (DP), collision energy and so on, were optimized in MS mode.

An API 3000 triple quadrupole mass spectrometer equipped with a MicroIonSpray source (Applied Biosystems/MDS Sciex) was employed to couple with a Crystal CE system (Thermo BioAnalysis, Franklin, MA, USA). The interface was constructed similarly in a three-layer coaxial arrangement (Fig. 1b). The sprayer tip was adjusted 20 mm away from the curtain plate and about 1 mm off-axis with an angle of 5–10 degrees to the ion-sampling orifice. The curtain plate was maintained at normal potential, i.e. 1 kV. A sheath flow of methanol was delivered

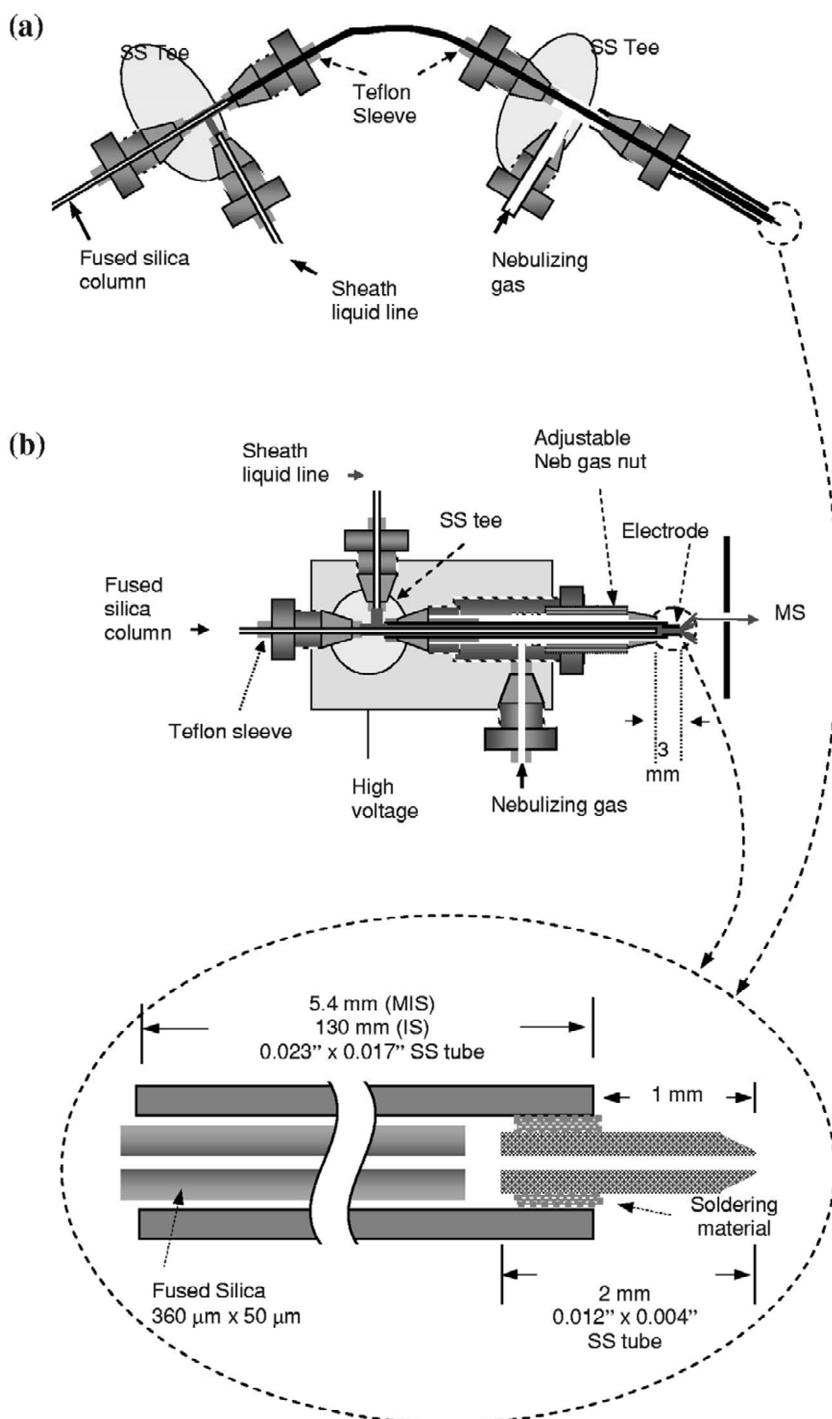

Fig. 1. Schematic diagram of the (a) IonSpray (IS) and (b) MicroIonSpray (MIS) sheath flow interface.

using a syringe pump. Samples or buffer solutions were infused through the sampling capillary using a second syringe pump for direct infusion study and for tuning purposes. Deprotonated molecular ions and product ions of compounds in negative mode were recorded; major MS parameters were optimized in multiple reaction monitoring (MRM) modes. Raw data were acquired and processed by Analyst software (Applied Biosystems/MDS Sciex).

3. Results and discussion

3.1. Electrode sprayer with step-down

The main goal of this study was to develop an electrospray interface that combines the high sensitivity feature of an ionspray interface and the high stability feature of a sheath-flow configuration for CE–MS on-line coupled analysis where a large size capillary column has to be used. In a conventional sheath-flow interface design, a stainless steel electrode tube has been commonly employed with an internal diameter of over 200 μm that is adequate for capillary columns of 185 μm O.D. to insert through and to protrude out [31]. A similar design has also been employed in which the CE column is pulled back 1 mm with reference to the electrode outlet [32], a terminal junction is formed at the exit of the column and serves as the CE terminal reservoir. It is obvious that this interface can be adapted for holding a large size column using a large stainless steel tube but reducing the terminal opening of the SS tube to achieve the sensitivity of a low-flow electrospray interface. One of the approaches was to incorporate a short step-down tube onto the large tube embracing the column.

The standard size of the step-down tube investigated is 100 μm I.D. and it operates at an optimal total flow-rate of 1 to 5 μl/min. Lower flow-rates require the use of smaller inner diameter stainless steel tubes that can be micro-fabricated.

3.2. Tuning of the interface

For tuning purposes, standards diluted in CE background electrolyte were infused through the column inlet by either a syringe pump at 0.2 μl/min or applying a CE inlet pressure of 5 p.s.i., while the sheath liquid was supplied typically at 2–3 μl/min (1 p.s.i=6894.76 Pa). It is worth noting that CE generates a typical electro-osmotic flow of hundreds of nanolitres per minute; therefore the bulk fluid being sprayed is from the sheath liquid such as methanol. Before running the experiment, solutions served as buffers and the sheath liquid was degassed. All source parameters and the sprayer positioning were optimized for high sensitivity and stability. As shown in Fig. 2a and b, the signal stabilities produced by hydrodynamic and electrokinetic infusion were 0.8% and 2.4% RSD, respectively. Singly and doubly charged peptide ions were observed in positive mode.

The concept of frontal chromatography was applied in the electrokinetic infusion experiment; samples were electrokinetically pumped into the CE column by applying constantly 30 kV at the inlet of the column. The two peptides were separated with two frontal electrophoretic signals (Fig. 3). This approach has been used to verify the righteousness of the interface set-up before sample analysis.

3.3. CE–MS of peptides

Fig. 4 shows the separation of angiotensin I and II. Three-period MS experiments in a run were performed. The first period was used during injection, ionspray voltage and nebulizer gas were set to zero to maintain the liquid drop at the sprayer tip, and to prevent siphoning. The last period was used to turn off the ionspray to keep the MS clean. The reproducibility is shown in Fig. 5. The RSDs are 1.3% and 4.7% for migration time and for peak height, respectively, with electrokinetic injections; they are 0.6% and 2.8%, respectively, with hydrodynamic injections.

3.4. Quantitative CE–MS–MS

Quantitation of nucleotides present at low levels is of extreme importance in studies of intracellular phosphorylation of antiretroviral nucleoside drugs. Fig. 6 shows a profile of 10^{-7} *M* nucleotide mixtures obtained by CE–MS–MS. Linear regression analysis suggested three orders of magnitude of dynamic range across a concentration range of 10^{-7} to 10^{-4}

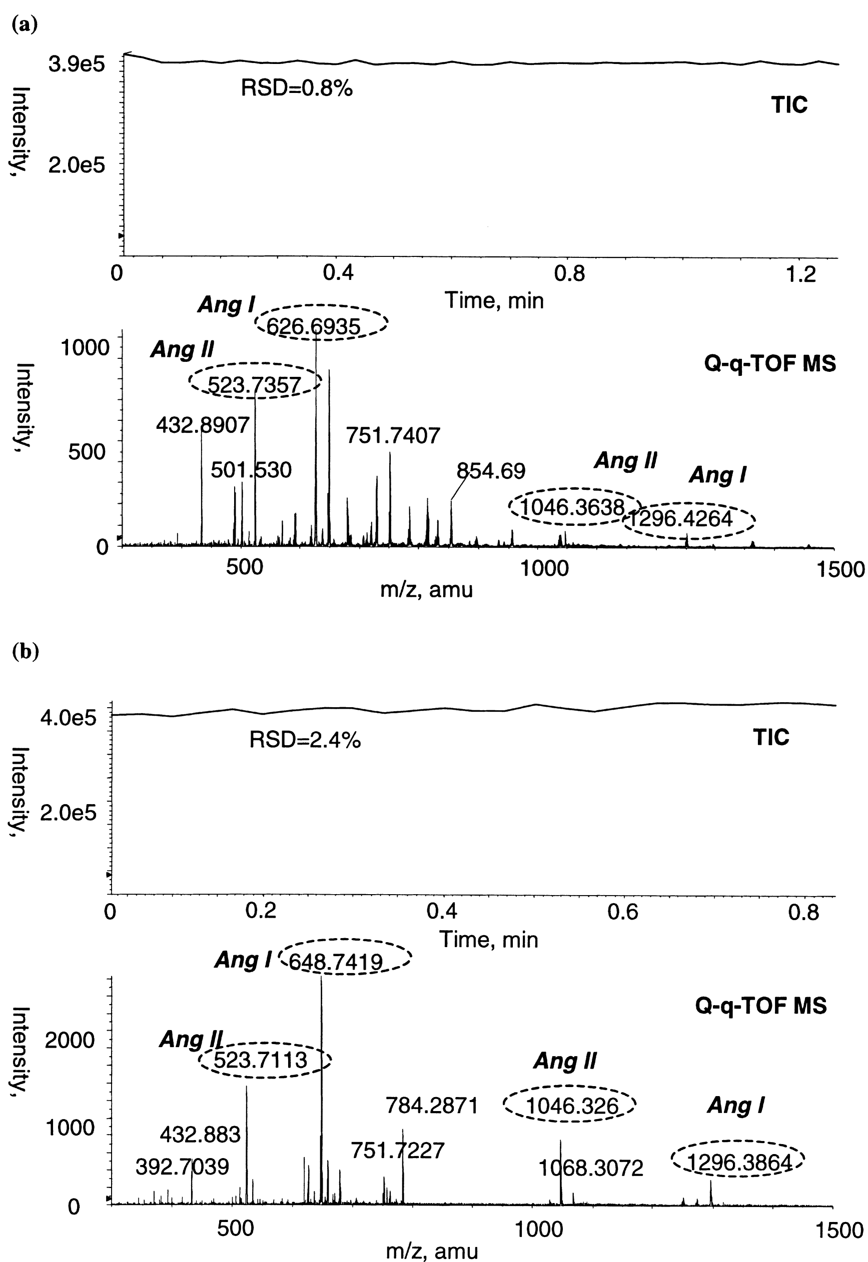

Fig. 2. Total ion current (TIC) and MS spectra of peptides infused (a) hydrodynamically or (b) electrokinetically to Q-q-TOF-MS using an ionspray sheath flow interface.

M with R^2 of 0.99 (Fig. 7). The limits of quantitation (LOQs) were elucidated from the analysis of the mixture of nucleotide standards, they were around 2, 1.5, 0.4 and 0.2 fmol for GTP, GDP, GMP and G, respectively.

4. Conclusion

A sheath flow CE–MS interface for coupling a large-size CE column and electrospray mass spectrometry has been developed and evaluated. This

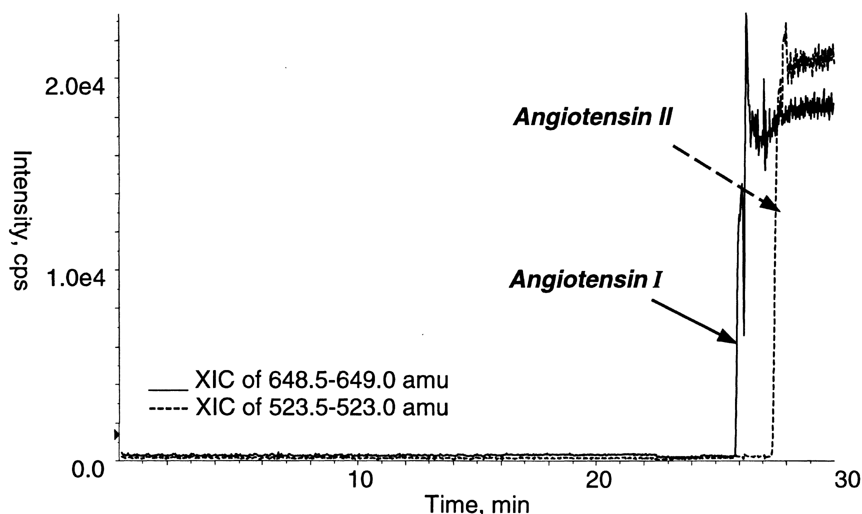

Fig. 3. Frontal electropherogram of peptides by CE–Q-q-TOF-MS using an ionspray sheath flow interface; 10^{-4} *M* peptides in 100 m*M* background electrolyte (BGE) with 30 kV electrokinetic pumping.

approach further extends the concept of sheath flow designs in CE–MS to achieve stable analytical performance over a long period of usage. Signal stability with an RSD of 0.8 to 2.4% was demonstrated by hydrodynamic and electrokinetic infusion analysis, and with a good CE–MS reproducibility in migration time (RSD less than 2%) and peak height (RSD less than 5%) of peptides. Sub-micromolar detectability of nucleotides by MS–MS with an LOQ of 0.2–2 fmol was routinely achieved with more than three orders of magnitude of linear response. Further studies on using a large metal tube with a sprayer tip

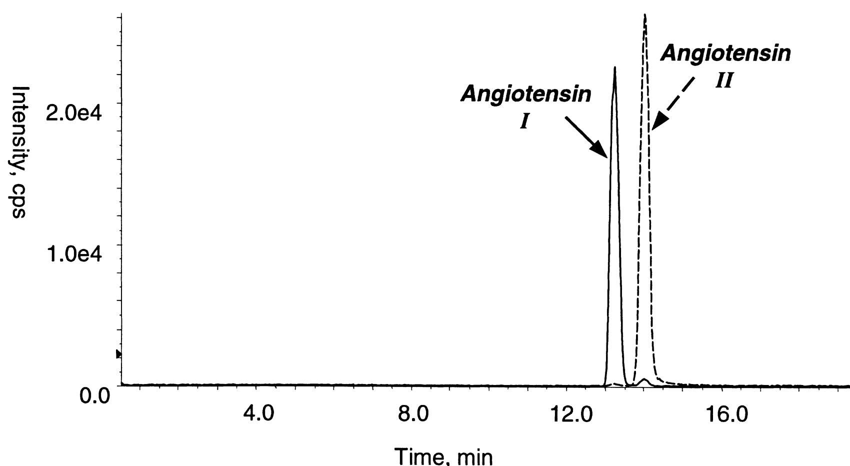

Fig. 4. Selected ion monitoring (SIM) electropherogram of peptides by CE–Q-q-TOF-MS using an ionspray sheath flow interface; 10^{-4} *M* peptides in 50 m*M* BGE with 2 p.s.i. hydrodynamic injection. Separations were performed at 25 kV with 0.2 p.s.i. inlet pressure in a fused-silica capillary 130 cm×50 μm×360 μm. The sheath liquid was delivered at 3 μl/min. Source conditions: 0–0.5 min, IS 0, nebulizer gas 0; 0.5–19.5 min, IS 5.2 kV, nebulizer gas 3; 19.5–20 min, IS 0, nebulizer gas 0. Curtain gas was set to 15. Declustering potential was 70 V with CAD (collision activated dissociation) gas 3.

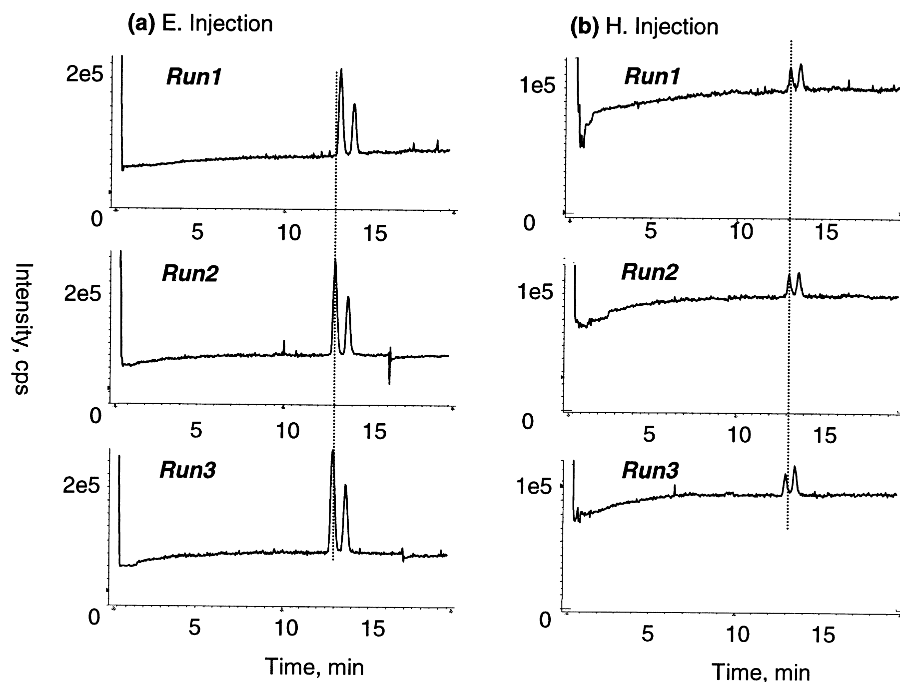

Fig. 5. TIC showing the reproducibility of CE–Q-q-TOF-MS using an ionspray sheath flow interface. Electrokinetic injections were made at 10 kV for 20 s, samples prepared in water; hydrodynamic injections were made at 2 p.s.i. for 10 s, samples were in buffer.

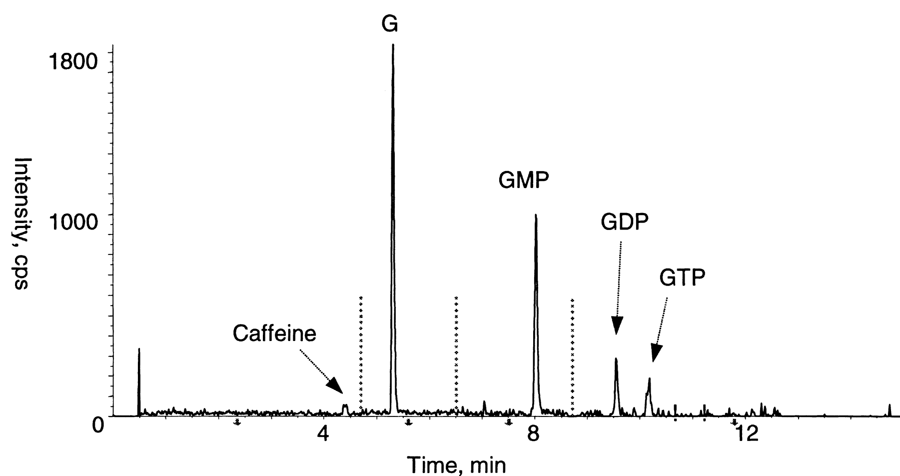

Fig. 6. MRM electropherogram of guanosine and its phosphorylated forms by CE–MS–MS on API 3000 with microionspray sheath flow interface. Each analyte was 10^{-7} *M* prepared in the background electrolyte, 10 m*M* NH_4Ac, pH 10.0. Samples were introduced at 100 mbar to the capillary column, 80 cm×50 μm×360 μm. Separations were performed at 375 V/cm. The sheath liquid was delivered at 3 μl/min. EOF was monitored using caffeine as a marker. Source conditions: MIS interface, −4.8 kV ionspray voltage with nebulizer gas of 1 and curtain gas of 10 applied. Deprotonated ions and MRM transitions (234/134, 282/150, 362/210, 362/79, 442/159, 442/150, 522/424, 522/159) were recorded with four-period experiments for fast speed scanning. The *S*/*N* values were 10.4 for GTP, 16.2 for GDP, 54.1 for GMP and 118 for G. The injection volume was around 20 nl.

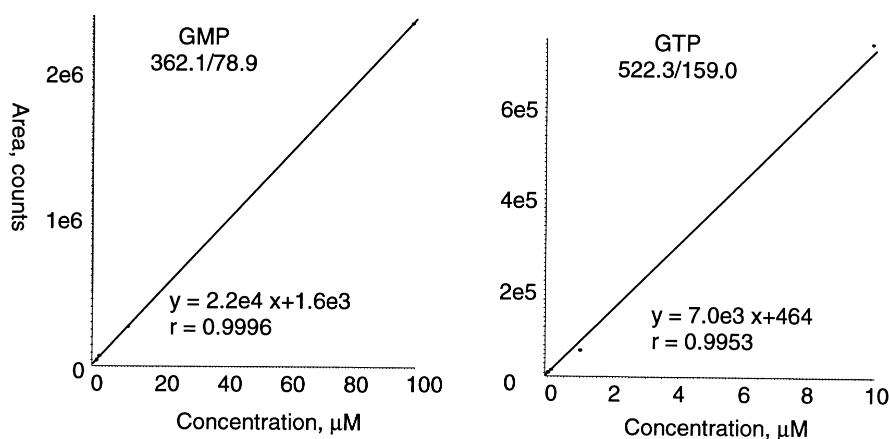

Fig. 7. Calibration curves of nucleotides by CE–MS–MS on API 3000 using MicroIonSpray sheath flow interface.

opening of smaller than 100 µm but big enough to prevent clogging are needed to assess its potential as a reliable and rugged interface for ultra-high stability and sensitivity analysis.

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