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Design and applications of a self-aligning liquid junction–electrospray interface for capillary electrophoresis–mass spectrometry

Timothy Wachs, Robin L. Sheppard[1], Jack Henion*

Analytical Toxicology, Diagnostic Laboratory, Cornell University, 927 Warren Drive, Ithaca, NY 14850, USA

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Abstract

A simple self-aligning liquid junction–electrospray interface for coupling a capillary electrophoresis (CE) system to an atmospheric pressure ionization (API) mass spectrometer (CE–MS) was developed. In contrast to previous liquid junction interfaces, the self-aligning liquid junction interface simplifies the precise alignment of the CE capillary and the sprayer needle and uses a positive make-up flow. Several capillary CE–MS applications were run using both the self-aligning liquid junction interface and the widely used sheath flow interface for comparison purposes. The electrospray stability of the self-aligning liquid junction interface is consistently better even when non-volatile electrolyte solutions are used. At first, some band broadening was obtained with the self-aligning liquid junction interface. Experiments with different CE buffer systems suggested that this band broadening was caused by the materials used in constructing the interface. By using a more inert material for the sprayer needle, the self-aligning liquid junction exhibits excellent electrophoretic resolution, comparable sensitivity, and higher signal-to-noise ratios when run under the same conditions as the sheath flow interface.

Keywords: Self-aligning liquid junction–electrospray interface; Ephedrine; Isoproternol; Orciprenaline; Terbutaline; Tulobuterol; Salbutamol; Clenbuterol; Ritodrine; Fenoterol

1. Introduction

Coupling a capillary electrophoresis (CE) system to an atmospheric pressure ionization (API) mass spectrometer (CE–MS) entails challenges that are more demanding than those for coupling a liquid chromatography system to a mass spectrometer [1]. Currently there are two "coupling" systems that are commonly in use. Olivares' et al. sheath flow interface [2] is generally easier to implement than Lee's et al. liquid junction interface [3]. The latter system requires a high degree of precision in aligning and spacing the CE capillary and the sprayer capillary, but when properly aligned provides excellent CE–MS results [4–9]. In contrast, the sheath flow approach can suffer from incomplete mixing of the sheath liquid and analyte which leads to an unstable ion current. A report comparing these two interfaces using CE with ion spray mass spectrometry found several disadvantages of the liquid junction including undesirable mixing of the CE electrolyte with the make-up liquid, increased band

*Corresponding author.

[1] On sabbatical from Xerox Corporation, 800 Phillips Road, 139-64A, Webster, NY 14580, USA.

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broadening and higher background noise level due to lower flow-rate of the make-up liquid [10]. It was also noted that the particular configuration of the liquid junction used did not allow for flow injection analyses (for calibration purposes) to be performed without disassembling the interface. This was due to the way the liquid junction was incorporated into the specific interface.

To develop an improved, more rugged CE–MS interface, the original design of the liquid junction interface was revisited. The first improvement was to deliver the make-up liquid at a controlled flow-rate instead of by gravity feed. The second improvement was to design an easy way for providing the precise alignment needed at the liquid junction. A simple, improved liquid junction interface designed for electrospray ionization (Fig. 1) was developed. The self-aligning liquid junction CE–MS interface incorporates the sprayer and the junction in a single unit. This new CE–MS interface addresses the four main problems that Pleasance et al. [10] reported with the original liquid junction: (1) Contamination of the make-up liquid when flushing the CE capillary with electrolyte or cleaning solutions is prevented, (2) band broadening due to the junction itself is minimized by optimizing the make-up flow-rate, (3) the background noise caused by unstable or low flow-rate of the make-up buffer is eliminated, and (4) flow injection analyses or calibrant infusion can easily be introduced via the side-arm of the Tee without disassembling the liquid junction. In order to evaluate the performance of the self-aligning liquid junction interface, several CE–MS applications were studied using both the new self-aligning liquid junction interface and the traditional sheath flow interface.

2. Experimental

Ammonium citrate was purchased form Aldrich (Milwaukee, WI, USA). (±)–Isoproterenol hydrochloride, terbutaline hemisulfate salt, salbutamol, clenbuterol hydrochloride, ritodrine hydrochloride, fenoterol hydrobromide and ammonium acetate were obtained from Sigma (St. Louis, MO, USA). Orci-

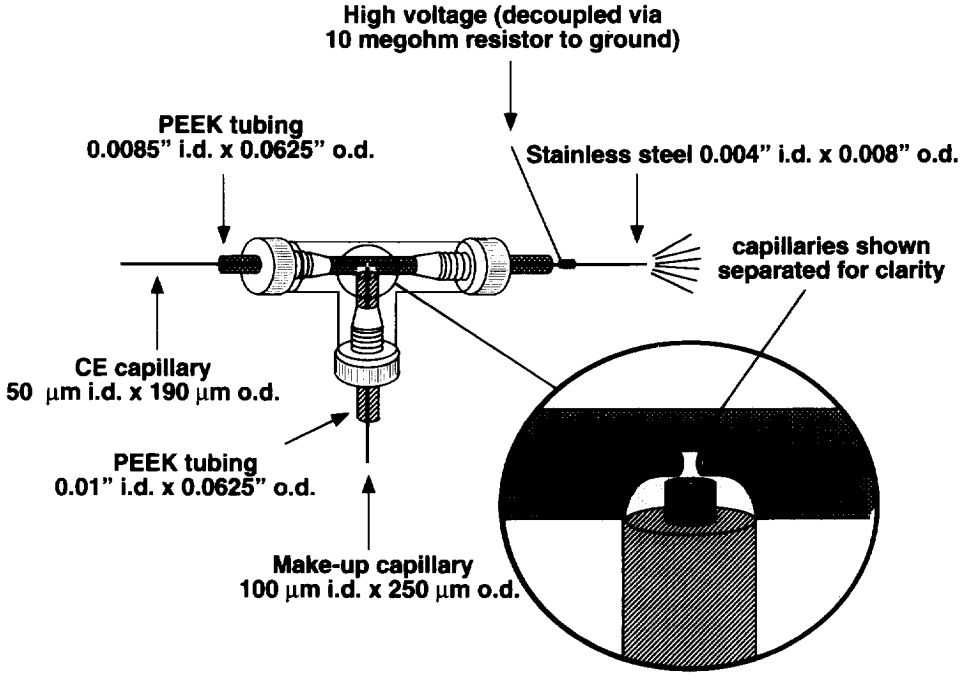


Fig. 1. Schematic diagram of the newly designed self-aligning liquid junction–electrospray interface for CE–MS. Shown with stainless steel sprayer needle. See text for benefits afforded by using fused-silica sprayer needle.

prenaline sulfate and tulobuterol hydrochloride were generous gifts from Dr. L. Leyssens at the Dr. L. Willems-Instituut (Diepenbeek, Belgium). Glacial acetic acid and methanol were obtained from Fisher Scientific (Pittsburgh, PA, USA). Water was purified with an in-house Barnstead Nanopure Ultrapure water system (Dubuque, IA, USA). Bond Elut Certify solid-phase extraction cartridges were obtained from Varian (Harbor City, CA, USA).

The new CE–MS interface shown in Fig. 1 was constructed using a 1/16″ PEEK Tee with 10–32 threads; 1/16″, 10–32 PEEK Fingertight Fittings and Ferrules or LiteTouch Ferrules; and 0.007″ I.D.×1/16″ O.D. and 0.0085″ I.D.×1/16″ O.D. PEEK tubing from Upchurch Scientific (Oak Harbor, WA, USA). Fused-silica capillary tubing was purchased from Polymicro Technologies (Phoenix, AR, USA). Stainless steel tubing was obtained from Small Parts (Miami Lakes, FL, USA).

The fused-silica CE capillary and the stainless steel sprayer capillary shown in Fig. 1 are held in a piece of PEEK tubing with an internal diameter closely matched to the outer diameters of the CE and sprayer capillaries. Prior to assembly, a notch or "window" is cut half-way into the PEEK tubing using a Dremel Tool grinding disc (Division of Emerson Electric Co., Racine, WI, USA). This exposes a 2–3 mm length of the internal channel of the PEEK tubing. The PEEK tubing with the window is held in a 1/16″ drilled out PEEK Tee with the window visible from the side arm of the Tee. The end of the stainless steel sprayer capillary is positioned in the center of the PEEK window and is then secured in place with a PEEK nut and ferrule. The CE capillary with a square-cut end is gently butted against the sprayer capillary and is tightened in place. It is not necessary to obtain a perfect square cut of the fused-silica capillary tubing since when the CE capillary is butted against the sprayer capillary, the imperfect butt connection provides the necessary gap for the make-up liquid to enter the junction. The width of the gap between the CE capillary and the sprayer capillary is not measured. If the connection is "perfect", the make-up liquid cannot enter the junction. However, in practice this seldom occurs. Once the sprayer capillary is positioned and secured, the CE capillary can be repeatedly removed and reinstalled by simply inserting it until it butts up against the sprayer capillary and then tightening the fitting. Earlier versions of this interface used PEEK fingertight fittings and ferrules. It was found that Upchurch LiteTouch ferrules used with ordinary stainless steel HPLC fittings were preferable since the PEEK tubing does not rotate or deform when these fittings are tightened, thus improving the durability of the interface. The make-up flow is provided by an auxiliary infusion pump (Harvard Apparatus Infusion Pump 22, South Natick, MA, USA) and is admitted through the side arm of the Tee. This provides a continuous sweep of the junction with the majority of the flow exiting through the sprayer capillary and a low but noticeable flow back into the CE capillary.

Solid-phase extraction of urine samples was performed using the Bond Elut Certify method for the extraction of phenylcyclidine form urine [11]. 5 ml of urine was mixed on a vortex mixer with 2 ml 0.1 *M* phosphate buffer, pH 6.0. Using a vacuum manifold, the Bond Elut extraction column was prepared by rinsing with 2 ml methanol followed by 2 ml 0.1 *M* phosphate buffer, pH 6.0. The diluted urine sample was then passed through the column. The column was rinsed with 1 ml 1.0 *M* acetic acid, dried under vacuum for 5 min, rinsed with 6 ml methanol, and dried under vacuum for 2 min. The sample was eluted with 2 ml 2% ammonium hydroxide in ethyl acetate, evaporated to dryness and reconstituted in 50 μl ethyl acetate.

Capillary electrophoresis was carried out using a Hewlett–Packard ^{3D}CE capillary electrophoresis instrument using bare fused-silica capillaries with dimensions of 50 μm I.D.×190 μm O.D. and ranging between 75 and 90 cm in length. Separations were not temperature controlled. Samples were loaded using pressure injection for 10 s at 50 mbar. The CE voltage was maintained between 23 and 30 kV, with an effective running voltage ranging between 18.5 and 27.5 kV when the electrospray needle voltage was maintained between 2.5 and 4.5 kV.

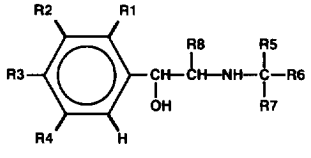
Mass spectrometry in the positive ion electrospray mode was performed on two different instruments: a Sciex (Thornhill, Ont., Canada) TAGA 6000E upgraded to an API III and a PE–Sciex API 300 system, both operated with unit mass resolution (0.5–0.6 amu peakwidth at half-height) in both quadrupole mass analyzers The instruments were

operated in either the selected ion mode (SIM) or selected reaction monitoring (SRM) mode. Dwell times of all SIM and SRM experiments were adjusted to obtain a 1 scan/s data acquisition rate. Data acquisition and presentation were performed using the standard Macintosh-based software provided by PE–Sciex. Electrospray ionization was used instead of ion spray ionization since it is more suited to the low flow-rates used with capillary electrophoresis as long as the make-up liquid contains sufficient organic solvent.

The sprayer voltage and sheath or make-up flow were not turned on until after each CE injection was completed. This was to ensure a reproducible injection volume regardless of the individual voltage and flow-rates used. The initiation of the electrospray caused any droplet of CE buffer on the end of the sprayer capillary to be expelled within 1 min of turning on the voltage. Therefore, the mass spectrometer data acquisition was initiated approximately 1 min after the CE run commenced in order to allow the ion current to stabilize before collecting the data.

3. Results and discussion

The structures of the drugs studied in this work are shown in Fig. 2. The majority of the β-agonists are used therapeutically as bronchodilators and/or as tocolytic agents. Some of the drugs, notably clenbuterol, have been shown to have growth promoting



Compound	$(M+H)^+$	R1	R2	R3	R4	R5	R6	R7	R8
Ephedrine	166	H	H	H	H	H	H	H	CH_3
Isoproterenol	212	H	H	OH	OH	H	CH_3	CH_3	H
Orciprenaline	212	H	OH	H	OH	H	CH_3	CH_3	H
Terbutaline	226	H	OH	H	OH	CH_3	CH_3	CH_3	H
Tulobuterol	228	Cl	H	H	H	CH_3	CH_3	CH_3	H
Salbutamol	240	H	CH_2OH	OH	H	CH_3	CH_3	CH_3	H
Clenbuterol	277	H	Cl	NH_2	Cl	CH_3	CH_3	CH_3	H
Ritodrine	288	H	H	OH	H	H	CH_3	CH_2-Ph-OH	CH_3
Fenoterol	304	H	OH	H	OH	H	CH_3	CH_2-Ph-OH	CH_3

Fig. 2. Structures of the β-agonists used in this work.

effects in meat animals [12–14]. However, the β-agonists have been banned by the European Community for use as growth promoters since they are toxic to humans at sufficiently high doses [15,16]. Therefore, the determination of the β-agonists in animal urine and tissue at low levels is currently an active area of research [17–19]. These compounds were selected as models in this study since the application of CE–MS could be beneficial in future regulatory aspects of these potential problems.

Before any CE separations were performed, infusion studies were carried out to optimize and compare the electrospray MS response for a selected compound with the conventional sheath flow interface and the new self-aligning CE–MS interface. A solution of 90 p*M* fenoterol in 20 m*M* acetic acid was electro-infused through the CE capillary at 10 kV in each case. A solution of 75:24:1 methanol–water–acetic acid was used for both the sheath liquid of the sheath flow interface and the make-up liquid of the self-aligning CE–MS interface. The flow-rates of the sheath and make-up liquid were optimized for maximum signal on each interface (2 μl/min and 1 μl/min respectively). A 5 mbar pressure was maintained on the CE inlet while using the self-aligning liquid junction interface to counter the small reversed flow of the make-up liquid into the CE capillary. The mass spectrometer was operated in the selected ion monitoring mode for the protonated molecule of fenoterol at m/z 304. The resulting ion current profiles indicated that the analyte signal for m/z 304 is higher by a factor of approximately 2.5 for the self-aligning liquid junction interface and the ion current signal is more stable than that obtained from a similar experiment using the sheath flow interface. The net result is a measured signal-to-noise ratio which is three times higher for the self-aligning liquid junction interface than the sheath flow interface.

As mentioned above, a positive CE inlet pressure is applied to counteract backward flow of the make-up liquid into the CE capillary when using the self-aligning liquid junction interface. When no inlet pressure is applied, the ion current signal can become unstable. Initially, inlet pressures of 5 to 10 mbar were used. However, in subsequent analyses it was found that a CE inlet pressure of only 1 mbar was necessary to counter the back pressure and to

stabilize the ion current signal. Any excess CE inlet pressure supplements the electroosmotic flow and results in a slightly faster analysis due to increased bulk liquid flow. If the electroosmotic flow-rate of a particular application is determined by CE–UV, the inlet pressure used with the self-aligning liquid junction for CE–MS can be optimized to produce the same effective electroosmotic flow. It should be noted that raising the level of the CE inlet in relation to the sprayer outlet could also provide the slight increase in flow that is needed to counter the back pressure from the make-up flow. It is usually more convenient and reproducible to program the CE instrument for the desired inlet pressure.

The CE–MS analysis used to compare the self-aligning liquid junction and the sheath flow interfaces was a separation of a synthetic mixture containing nine β-agonists. A standard solution (about 0.1 p*M* of each compound injected) was separated on a 50 μm×90 cm capillary using 10 m*M* ammonium acetate/10 m*M* acetic acid at 30 kV and 5 mbar inlet pressure. The TAGA/API III mass spectrometer was operated in the selected ion monitoring mode for the protonated molecules at m/z 166, 228, 212, 226, 277, 240, 288 and 304. The β-agonist compounds are very similar structurally (Fig. 2) so that some of them are difficult to resolve completely under the CE conditions used. Isoproterenol and orciprenaline, which both have a molecular mass of 212, were not resolved under these experimental conditions. Therefore, only eight peaks are visible in the following experiments. Methanol at 4 μl/min was used for both the sheath liquid and the make-up liquid in the respective interfaces. It was found that when peak resolution is an issue, as experienced in this separation, a higher make-up flow in the self-aligning liquid junction can help minimize band broadening. Still, the initial selected ion current electropherograms from the self-aligning liquid junction shown in Fig. 3A display more band broadening than is observed in the sheath flow electropherograms shown in Fig. 3B. The electropherogram peaks are between 20 and 115% wider at half-height in the case of the self-aligning liquid junction (Fig. 3A). The signal-to-noise ratios of the peaks for the self-aligning liquid junction are similar or worse than for the sheath flow interface. Since the self-aligning liquid junction had performed much better in other applications (unpublished results), it was reasoned that further improvement could be made with the β-agonist separation.

While there are many examples of successful use of the original liquid junction CE–MS interface [3–9], the band broadening that was observed for the β-agonists in the self-aligning liquid junction was also evident in several other reports where liquid junction interfaces were used [10,20]. In order to investigate this problem further, CE–MS experiments using in-line ultraviolet (UV) detection at 200 nm were performed using the β-agonist separation. These experiments allow one to simultaneously monitor the CE separation characteristics before and after the analytes pass through the self-aligning liquid junction interface. Comparisons of ammonium acetate and ammonium citrate CE buffers demonstrated that the analyte peak widths were significantly improved when citrate was used (data not shown). The ammonium citrate apparently minimized band broadening caused by the self-aligning liquid junction under these experimental conditions. This unexpected result suggested that sample adsorption or interaction was occurring on the inner walls of the self-aligning liquid junction and that this interaction could be reduced by using ammonium citrate instead of ammonium acetate as the CE electrolyte. The possibility of sample adsorption prompted a closer look at the materials used for constructing the new interface.

Since the materials in the self-aligning liquid junction used above consist of only PEEK, fused-silica and stainless steel, substantial adsorption or interaction of the β-agonists was suspected to be occurring with the stainless steel sprayer. The stainless steel sprayer needle was replaced with a fused-silica capillary needle of the same I.D and O.D. as the CE capillary. In this configuration, the electrospray voltage was applied to a short stainless steel tube which couples the make-up line to the interface tee. This modification is shown in Fig. 4 and can be compared to the schematic diagram in Fig. 1. The results shown in Fig. 3C are a dramatic improvement in the CE–MS separation efficiency. Fig. 3C shows the CE–SIM-MS selected ion current electropherograms of the nine β-agonists using 10 m*M* ammonium acetate–10 m*M* acetic acid electrolyte solution and the self-aligning liquid junction with the fused-silica

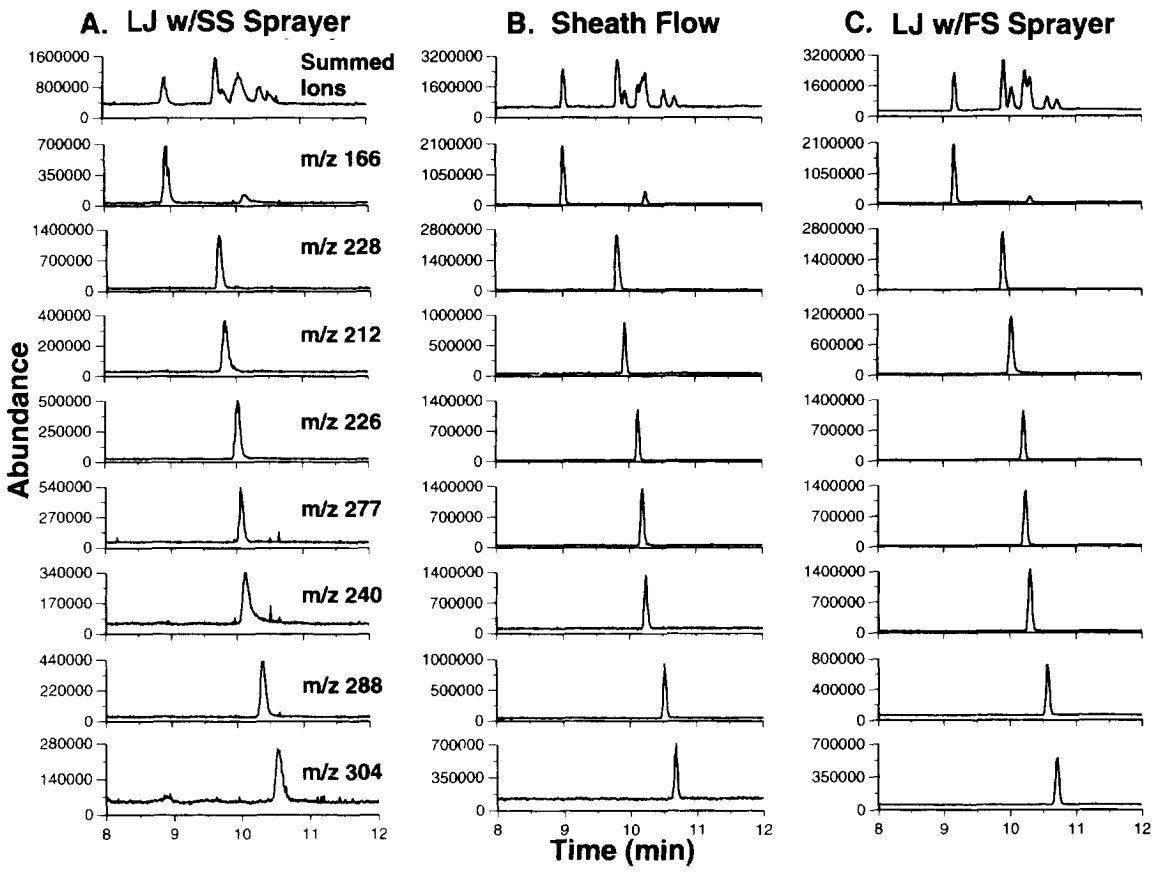


Fig. 3. CE–SIM-MS separation of a mixture of nine β-agonists monitoring the protonated molecules of the drugs at m/z 166, 212, 226, 228, 240, 277, 288 and 304. The summed ion current and the individual selected ion current electropherograms are shown. The results from the self-aligning liquid junction (LJ) interface with a stainless steel sprayer (A), the sheath flow interface (B), and the self-aligning liquid junction interface with a fused silica sprayer (C) are shown. CE–MS conditions: 10 m*M* ammonium acetate–10 m*M* acetic acid, 30 kV with 10 mbar inlet pressure for (A), 30 kV with 5 mbar inlet pressure for (B), 30 kV with 30 mbar inlet pressure for (C); methanol make-up and sheath liquid at 4 μl/min in (A) and (B); 2 m*M* ammonium acetate in methanol make-up at 0.5 μl/min in (C).

sprayer needle. When compared to Fig. 3A in which the same CE conditions and the stainless steel sprayer needle were used, the improvement afforded by the fused-silica sprayer is readily apparent. Overall, the calculated peak resolution for the close-migrating peaks ranged from 1 to 5 for the stainless steel sprayer needle in Fig. 3A and from 1 to 53 for the fused-silica sprayer needle in Fig. 3C. The calculated peak efficiencies for the fused-silica sprayer needle were increased by 1.1 to 3.2 times over the stainless steel sprayer needle. In addition, when compared to the sheath flow interface results shown in Fig. 3B, the resulting signal-to-noise ratios with the self-aligning liquid junction are on average 3.3 times higher in Fig. 3C. The sensitivity of the self-aligning liquid junction with the fused-silica sprayer needle (Fig. 3C) is now comparable to the sheath flow interface (Fig. 3B). With baseline peak widths that average 9 s for the sheath flow interface and 9.6 s for the liquid junction interface under identical CE buffer conditions, it can be shown that no significant band broadening is caused by the self-aligning liquid junction.

In order to show a "real" sample analysis application, the self-aligning liquid junction interface was used to analyze extracts of a urine sample spiked with six of the β-agonists shown in Fig. 2. Human urine was spiked at 50 ng/ml for each drug. The

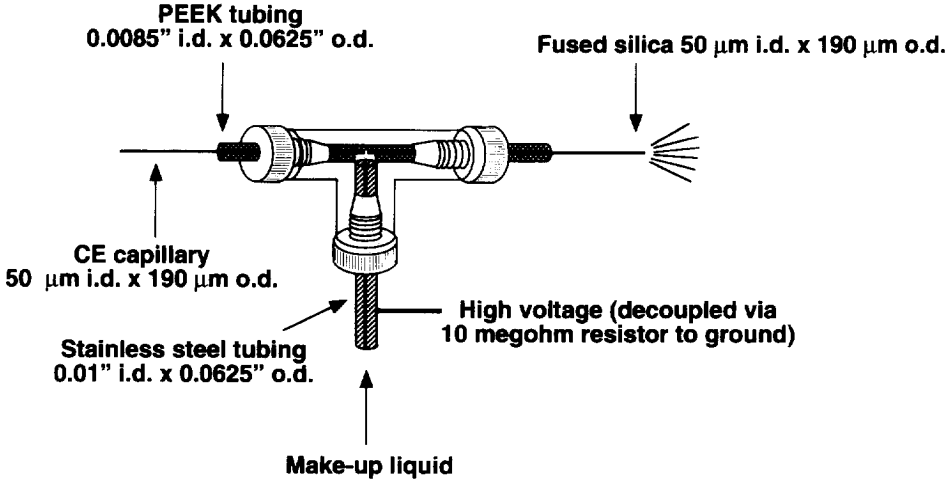


Fig. 4. Schematic diagram of self-aligning liquid junction CE–MS interface with a fused-silica sprayer needle instead of stainless steel.

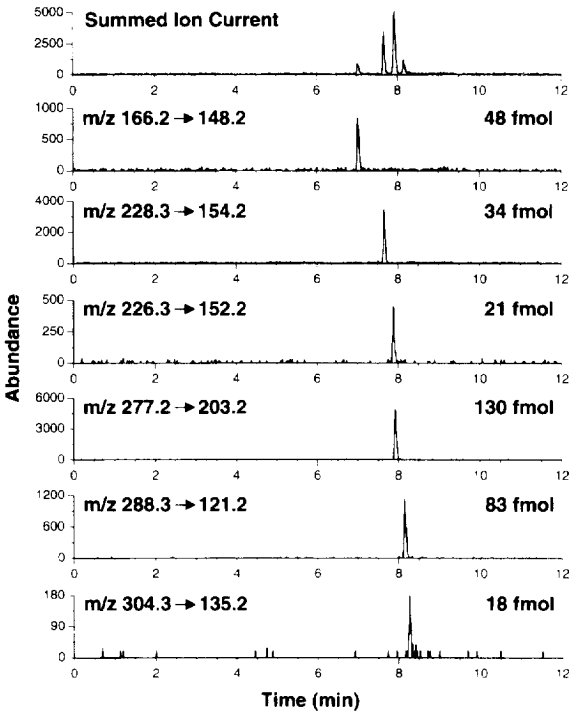


Fig. 5. CE–SRM-MS of urine extracts spiked with six β-agonists using 10 m*M* ammonium acetate–10 m*M* acetic acid electrolyte solution with the self-aligning liquid junction. The ion current profile of each precursor–product transition is shown individually and is labelled with the calculated injection amount. CE–MS conditions: 30 kV with 5 mbar inlet pressure, methanol make-up liquid at 4 μl/min.

analytes were isolated from the urine by solid-phase extraction on Bond Elut Certify cartridges using the Bond Elut procedure for extracting phenylcyclidine [11]. The extract from 5 ml of urine was evaporated to dryness and reconstituted in 50 μl ethyl acetate. This sample was analyzed by CE–MS–MS on the Sciex API 300 in the selected reaction monitoring (SRM) mode for the six precursor-product ion transitions shown in Fig. 5. The shorter migration times observed in this experiment are due to a shorter CE capillary (75 cm instead of 90 cm) than was used in Fig. 3. The injected amounts are calculated to range from 18 to 130 f*M* based on external standards. The combination of the solid-phase extraction sample clean-up and the selectivity of using MS/MS results in an electropherogram free of matrix interferences. Although these detection limits are not as low as reported earlier [17,18,21], it is believed that improved detection limits may be achieved in the future using API ion trap [22] and time-of-flight mass spectrometers [23].

4. Conclusions

This report describes an improved version of the liquid junction CE–MS interface which is simple to assemble and align and is analytically more rugged for extended use. The self-aligning liquid junction–electrospray interface provides improved mixing of

the CE effluent and the make-up liquid which leads to better spray stability and improved signal-to-noise ratio. Head-to-head comparisons with the traditional sheath flow interface demonstrate that the self-aligning liquid junction provides about three times higher signal-to-noise ratios. Initial experiments showed varying degrees of band broadening when using the self-aligning liquid junction interface. The electrophoretic performance of liquid junction type interfaces have often shown losses in resolution when compared to sheath flow interfaces. Closer examination of the sources of the band broadening indicated that the construction materials in the interface were a significant factor to the cause. The results reported here show that at least in the case of the β-agonist samples, the majority of the band broadening was contributed by adsorption and/or interaction of the analytes with the stainless steel sprayer needle. This band broadening can be remedied by using electrolyte solutions which help minimize adsorption, but the source of the problem can be eliminated by replacing the stainless steel needle with a fused-silica or other inert sprayer needle material. The end result is a CE–MS interface which is simple, rugged, extremely stable and which provides comparable sensitivity and higher signal-to-noise ratios than the widely used sheath flow interface.

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References

[1] E.C. Huang, T. Wachs, J.J. Conboy and J.D. Henion, Anal. Chem., 62 (1990) 713A–725A.
[2] J.A. Olivares, N.T. Nguyen, C.R. Yonker and R.D. Smith, Anal. Chem., 59 (1987) 1230–1232.
[3] E.D. Lee, W. Mück, J.D. Henion and T.R. Covey, Biomed. Environ. Mass Spectrom., 18 (1989) 844–850.
[4] J.M. Johansson, R. Pavelka and J.D. Henion, J. Chromatogr., 559 (1991) 515–528.
[5] J.M. Johansson, E.C. Huang, J.D. Henion and J. Zweigenbaum, J. Chromatogr., 554 (1991) 311–327.
[6] E.D. Lee, W. Mück, J.D. Henion and T.R. Covey, J. Chromatogr., 458 (1988) 313–321.
[7] E.D. Lee, W. Mück, J.D. Henion and T.R. Covey, Biomed. Environ. Mass Spectrom., 18 (1989) 253–257.
[8] F. Garcia and J.D. Henion, Anal. Chem., 64 (1992) 985–990.
[9] F. Garcia and J.D. Henion, J. Chromatogr., 606 (1992) 237–247.
[10] S. Pleasance, P. Thibault and J. Kelly, J. Chromatogr., 591 (1992) 325–339.
[11] Varian Sample Preparation Products, Harbor City, CA, USA, Bond Elut Certify Instruction Manual.
[12] J.P. Hanrahan, β-Agonists and their Effects on Animal Carcass Quality, Elsevier, London, New York, 1987.
[13] Y.T. Yang and M.A. McElligot, Biochem. J., 261 (1989) 1–10.
[14] P.J. Reeds and H.J. Mersmann, J. Anim. Sci., 69 (1991) 1532–1550.
[15] R.W. Jones, R.A. Easter, F.K. McKeith, R.H. Dalrymple, H.M. Maddock and D. Betchel, J. Anim. Sci., 61 (1985) 905–913.
[16] P.K. Baker, R.H. Dalrymple, D.L. Ingle and C.A. Ricks, J. Anim. Sci., 59 (1984) 1256–1261.
[17] C.T. Elliott, J.D.G. McEvoy, W.J. McCaughey and D.H. Shortt, Analyst, 118 (1993) 447–448.
[18] C.T. Elliott, W.J. McCaughey and H.D. Shortt, Food Addit. Contam. 10 (1993) 231–244.
[19] B. Stoffel and H.H.D. Meyer, J. Anim. Sci., 71 (1993) 1875–1881.
[20] N.J. Reinhoud, W.M.A. Niessen, U.R. Tjaden, L.G. Gramberg, E.R. Verheij and J. Van der Greef, Rapid Commun. Mass Spectrom., 3 (1989) 348–351.
[21] J. Cai and J.D. Henion, Multi-Residue Method for beta-Agonists by Immunoaffinity Extraction-LC/LC/MS/MS, in Proceedings of The 43rd ASMS Conference in Mass Spectrometry and Allied Topics, ATLO-Ta, GA, 1995.
[22] J. Henion, A. Mordehai and J. Cai, Anal. Chem., 66 (1994) 2103–2109.
[23] D.M. Lubman and M.G. Qian, Anal. Chem., 67 (1995) 234A–242A.