Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1053 (2004) 37–42
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Review

Sheathless electrospray ionization interfaces for capillary electrophoresis–mass spectrometric detection Advantages and limitations

Haleem J. Issaq*, George M. Janini, King C. Chan, Timothy D. Veenstra

Laboratory of Proteomics and Analytical Technologies, SAIC Frederick Inc., National Cancer Institute at Frederick, P.O. Box B, Frederick, MD 20702, USA

Available online 17 September 2004

Abstract

A review of sheathless interfaces for capillary electrophoresis (CE)–mass spectrometry (MS) is presented. The review discusses the on-line CE–MS system requirements, advantages and weaknesses of current sheathless interface designs for CE–electrospray ionization MS, and comparison between sheath flow and sheathless interfaces. The advantages and limitations of three sheathless designs are discussed and commented upon, these include single-capillary, two-capillary and three-piece designs.

Keywords: CE/ESI-MS; CE interface; Sheathless CE interfaces; CE/MS

Contents

1. Introduction

Capillary electrophoresis (CE) is an excellent microseparation technique that has been used for the separation of ions, small molecules such as amino acids to large biomolecules such as peptides, proteins and nucleic acids. Detection in CE is usually carried out on-column using ultraviolet (UV) absorbance or laser-induced fluorescence (LIF). Unfortunately, UV detection is not very sensitive, while LIF might require solute derivatization with a fluorescent tag. Also, UV, electrochemical and LIF detection does not provide the information necessary to directly determine the structure of the detected solute. On the other hand, mass spectrometry (MS) is not only a sensitive detection technique, it can also be applied for the detection of a wide range of analytes without derivatization and gives the information necessary to determine the structural formula of the analytes of interest. Therefore, the

* Corresponding author. Tel.: +1 3018467186; fax: +1 3018466037.
E-mail address: issaqh@mail.ncifcrf.gov (H.J. Issaq).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.08.116

marriage of both instrumental analytical techniques would result in an extremely powerful tool for the separation, identification, and characterization of a wide range of molecules. The on-line interfacing of CE with MS, however, is not as easy as that of CE–UV or CE–LIF where on column detection is carried out. As a matter of fact, the construction of a reliable CE–MS system is not a trivial matter because of the requirements of both the CE continuous electrical circuit for electrophoretic separation and the mass spectrometer electrical contact for efficient electrospray ionization. The running buffers normally used in CE, for example sodium phosphate or borate, are not MS compatible (low volatility) and need to be replaced with volatile buffers such as ammonium formate or ammonium acetate. This substitution, however, may affect the quality of the separation. Although CE gives high resolution, the concentration detection limits are not very high, however the use of on-column concentration can help alleviate such a problem [1]. The challenge is how to couple on-line CE to an MS and achieve electrical continuity that will allow uninterrupted operation of both systems, and not affect the quality of separation or the detection sensitivity. Electrospray ionization (ESI) is the ideal method for on-line interfacing of CE to MS because it facilitates the transfer of analytes from the liquid phase of the CE to the gas phase of the MS. Three interface designs have been developed in the last 17 years for coupling CE with MS. The first CE–MS interface, coaxial sheath flow, was first introduced by Smith and his group in 1987 [2] and improved upon in later work [3]. Coaxial sheath flow is formed using two concentric metal capillaries whereby the CE terminus and the makeup flow line are inserted into the inner capillary. Delivery of the sheath gas or liquid is through the space between the inner and outer capillaries. Two years later, Henion and coworkers [4] introduced the liquid junction interface where a tee forms a junction between the CE terminus and a makeup flow line that transfers the resolved analytes to the ion source of the MS. Smith and his coworkers [5] realized the limitations of sheath liquid flow and developed a sheathless interface. Since then there have been many improvements on the design of these three interfaces [6–8]. This review traces the development of sheathless interfaces since they were first introduced in 1994 and comments on the advantages of each design and its inherent and practical limitations.

2. On-line CE–electrospray ionization MS system requirements

An on-line CE–ESI-MS system is made of a CE instrument, a mass spectrometer, an interface that will allow the transfer of analytes from the CE column outlet to the MS source, a closed CE electrical circuit for electrophoretic separation of the analyte mixture and a closed electrical circuit for the generation of continuous, stable and uniform fine spray stream that affords sensitive MS detection. The coupling of CE to MS has been a challenging problem because an ideal interface is one that is constructed in such a way that the CE separation column and the spray tip form a single continuous unit in order to eliminate any dead volume that may lead to diffusion and affect the quality of separation. In addition, the design should preserve the electric circuits of both the CE system and the spray tip. Also, it would be advantageous if no external solvent is added to the system (sheath liquid) that dilutes the analyte concentration and affects the detection sensitivity.

3. Sheath versus sheathless interfaces

The advantages of sheathles interfaces as compared to interfaces that require a sheath flow have been discussed in various publications [5–8] and will be mentioned here briefly. The main difference between these designs is that sheathless interface does not require the external flow of a coaxial sheath liquid to establish electrical contact with the CE effluent to facilitate the electrospray process. The disadvantage of sheath liquid introduction is the dilution of the analytes resulting in lower detection limits. Kelly et al. [6] compared both interfaces and concluded that sheathless gave a 10-fold increase in sensitivity. It is a fact that one of the weaknesses of CE is that although the mass detection levels are quite high the amount of the injected sample is low. Therefore, dilution of the analytes by sheath liquid is not a desirable approach. Also, constructing an interface with sheath flow is more complex than constructing a sheathless interface, as will be discussed later. In principle, the sheathless interface is the most optimal design for coupling CE on-line with ESI-MS because of the compatible flow rates of each technique, which leads to high desorption and ionization efficiencies and, consequently, high sensitivity. In contrast to the sheath flow interface, analytes are not diluted and no additional fluids are present at the spray tip other than the CE column effluent.

4. Sheathless design strategies

A variety of sheathless designs have been introduced in the last decade that satisfy the requirement of closing the CE separation capillary circuit while simultaneously providing an electrical potential to the spray tip. These include the use of (a) single capillary, (b) two capillaries, and (c) three capillaries. Each of these will be discussed separately.

4.1. Single-capillary designs

There have been numerous approaches to interfacing CE with MS using a single-capillary format whereby uninterrupted electrical contact required for capillary zone electrophoresis (CZE) and ESI was established by different means [5–31]. Wahl et al. [5] introduced the first single capillary-sheathless interface. They coated the capillary outlet with a conductive metal (i.e. silver deposition) at the cathode end that served to define the CE field strength along the

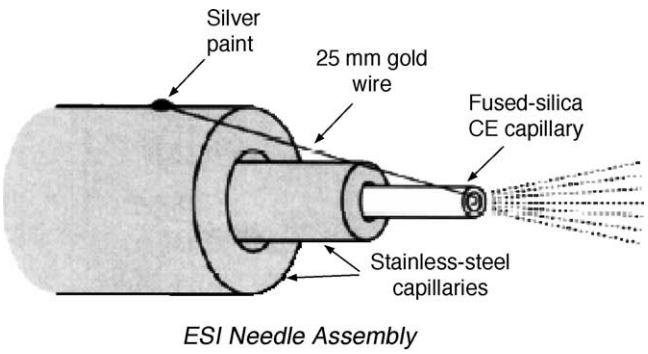

Fig. 1. CE–ESI interface (reprinted with permission from reference [16]).

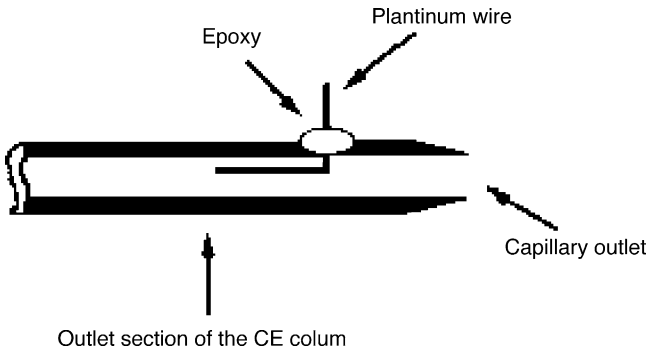

Fig. 2. Schematic diagram of the in-capillary electrode interface for CE–ESI-MS showing the outlet of the CE column (from Ref. [17] with permission).

capillary column and also provide the necessary electrospray potential. Such an arrangement did not interfere with the CE separation process. Normally the tips of the capillaries are tapered or sharpened before coating to increase the electric field strength. This can be achieved by etching the tip with hydrofluoric acid, mechanically grinding the tip or by heating and pulling. Since this work, a number of studies reported on the use of similar strategies for coating the cathodic end of the capillary with a metal [6–16], polymer [17], or carbon [18]. Although these approaches gave good results they were not durable, lasting only a few hours to a few days because the coatings are mechanically unstable. However, the stability of the metal coating was increased by reinforcing it with silanes [13], SiOx [23], or chromium [24] under the conducting metal layer, or by ink under the conducting carbon layer [18].

Fang et al. [16] used a different approach; they inserted a thin gold wire (25 μm) into the outlet of the capillary. They modified a commercial ESI unit by replacing the original inner stainless steel needle with the fused silica capillary column in the needle assembly. One end of the wire (about 2 mm) was inserted into the cathode end of the capillary while the other end of the wire was connected to the needle assembly with silver paint to establish the capillary potential Fig. 1 [16]. This arrangement worked well for the authors because they used a 100 μm capillary column. This arrangement may not be acceptable if a narrower internal diameter capillary is used.

Cao and Moini [17] constructed a sheathless interface by inserting a 25 μm diameter platinum wire, approximately 0.2–0.3 cm, into a pinhole that was drilled, about 2 cm from the outlet of a 75 μm i.d. × 150 μm o.d. fused silica capillary (Fig. 2). The wire was kept in place by covering the hole with epoxy. In this design the platinum wire electrode acts as both the low voltage electrode of the CE electric circuit and as a connection for the ESI voltage. According to the authors the CE current, 2.5 μA ± 0.25 μA, was stable for the maximum time studied (i.e. 50 min). Such an interface in which a wire is inserted inside the separation capillary, however, may generate turbulence that may affect the resolution in addition to being difficult to construct.

Petersson et al. [21] used a different approach for interfacing CE with ESI-MS. High voltage is applied at the outlet of the separation capillary by a stainless steel tube (a liner) through which the capillary is drawn. A compensating current between the liquid and the liner is maintained by a natural liquid film, which is built up at the outer surface of the capillary end i.e., the capillary effluent is split at or near the capillary outlet to fill the gap between the capillary and the outer coaxial metallic sleeve. They concluded that the sensitivity can be increased several fold if the capillary is mounted close to and perpendicular to the sampling cone in a counterflow of heated nitrogen gas. Good results were achieved by employing a narrow separation capillary with a sharpened end that was drawn through a sharpened liner and extended 50 μm. Also, required is the use of 20–30% (v/v) organic modifier and a CE field over the separation capillary that is at least 1 kV. All these requirements do not make this interface a simple one to use.

In another approach, a sheathless and electrodeless microelectrospray interface for the online coupling of CZE to MS was developed whereby electrical continuity is achieved by adjusting the position of the outlet of the capillary such that electrical contact is established through the air to the grounded inlet capillary of the MS [22]. The outlet of the fused silica CZE capillary is tapered and serves as the microspray tip. The spray does not contain a sheath flow nor does the capillary tip contain an electrode or a conductive coating. The capillary tip is placed directly in front of the MS orifice. The electrical field for the CZE separation as well as for the electrospray is delivered by the CZE high-voltage power supply. The electrical contact at the capillary outlet is established through the air between the spray tip and the inlet of the mass spectrometer, which is at ground potential A detection limit of 5 nmol/L was reported. The advantage of such an interface is that it does not affect the quality of the separation. However, the position of the spray outlet (column) is critical and improper placement of the spray tip with respect to the MS orifice can negatively influence solute ionization and ultimate sensitivity. As well as the separation since the applied voltage is split between the liquid in the capillary and the ESI process.

Our laboratory introduced a rugged and simple sheathless interface for CE–MS where the separation column, an electrical porous junction, and the spray tip are integrated within a single piece of a fused silica capillary [25]. ESI is

accomplished by applying an electrical potential through an easily prepared porous junction across a 3–4-mm length of fused silica. A stable electrospray is produced at nanoflow rates generated in the capillary by electrophoretic and electroosmotic forces. The interface is particularly well suited for the detection of low-femtomole levels of proteins and peptides. The ruggedness of this interface was evident by the continuous operation of the same column for over a 2-week period with no detectable deterioration in separation or electrospray performance. In this design, spray tips were made by applying heat from a microtorch while pulling gently. The resulting long tapered tip is later trimmed to the desired tip inside diameter using a glass tube cutter. The polyimide coating was removed from a 3–4-mm section of the capillary at a distance of 5 cm from end of the spray tip and the capillary was trimmed to a total length of 60 cm. Electrical conductance between the solute within the capillary and the anode buffer was constructed by etching the exposed section of the capillary with hydrofluoric acid (HF) to reduce its outside diameter (o.d.) without affecting the i.d. Over the course of the reaction (∼6 h), the capillary wall thins to about 15–20 μm. Although this porous junction created by HF etching is fragile, the capillary is firmly held inside the reservoir protecting it from breaking. The reservoir also serves to contain the buffer for closing the CE circuit and providing the spray voltage, Fig. 3.

In a later design, the capability for on-column sample enrichment was added into the sheathless interface, Fig. 3 [26]. A miniaturized solid phase extraction cartridge, made of reversed-phase material was attached to the CE capillary near the injection end as shown in Fig. 3. Capillaries with different inside and outside diameters were evaluated to optimize the performance of the CE–MS system, resulting in a mass limit of detection of 500 amol for tandem MS analysis of a standard peptide using a 20 μm i.d. capillary. The improved design incorporates an efficient method to preconcentrate a sample directly within the CE capillary followed by its electrophoretic separation and detection using a true zero dead-volume sheathless CE–MS interface.

In another approach Wahl and Smith [31] developed an interface whereby the electrical connection was made by constructing a micro-hole 2 cm from the end of the separation capillary. The micro-hole was then sealed with conductive gold epoxy that served as an electrical contact. Such a design is cumbersome and it is not easy to construct.

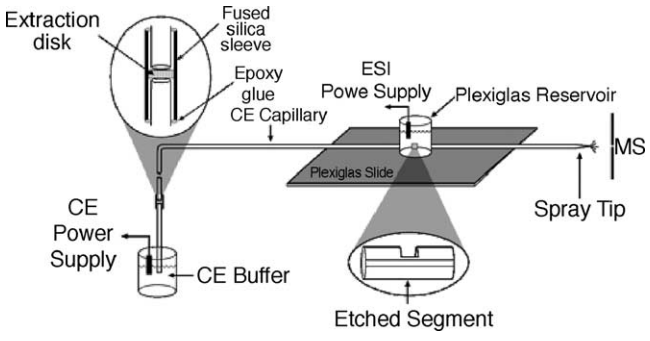

Fig. 3. Schematic diagram of the CE capillary with on-column. Microsolid phase extraction cartridge-sheathless ESI-MS interface (from Ref. [26] with permission).

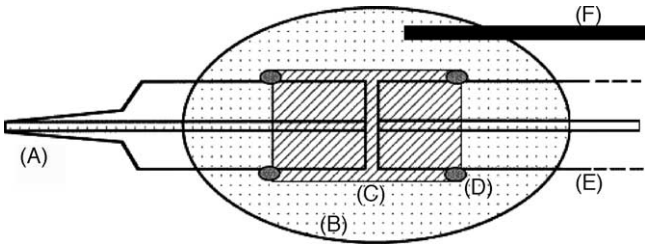

Fig. 4. Schematic diagram of a microdialysis CE–ESI-MS interface (from Ref. [32] with permission).

4.2. Two-capillary designs

Another strategy for the fabrication of a sheathless interface is to use two pieces of capillary whereby the CE separation capillary is connected to a short spray tip via a sleeve. The sleeve could be a piece of microdialysis tubing, [32] stainless steel tubing [33], or a micro-tee [34]. In the design of Severs and Smith [32] a separation capillary and 2 cm long ESI emitter capillary were butted together inside a 1.5 cm length of dialysis tubing, and epoxy was then applied around the outside of the dialysis tubing/capillary boundaries, as illustrated in Fig. 4. After the epoxy had dried, the capillary was inserted through a 250 μL Eppendorf pipet tip containing an electrolyte identical to that employed in the separation. The use of an open reservoir rather than an enclosed/limited reservoir, and plastic rather than metal, avoids problems due to gas bubbles in the liquid circuit. The pipet tip was connected to an *x–y–z* motion manipulator for positioning relative to the ion sampling orifice, and a copper wire was inserted in the electrolyte reservoir and connected to a high-voltage power supply A common ground connection was also formed between the CE system, the shielding of the ESI high-voltage power supply lead cable, and the triple quad MS system.

Tong et al. [34] developed a sheathless liquid-metal junction interface for CE–ESI-MS–MS. Approximately 2–3 mm length of polyimide coating was removed from a piece of coated capillary (∼5–10 cm long), and the exposed fused-silica tip was etched in 48–51% HF while purged with helium at 400 psi (1 psi = 6894.76 Pa). After the exposed fused silica was etched to a tip, the polyimide coating was further burned to expose ∼3 mm of the tapered tip. The total length of the tip was then trimmed to 3 cm. The butt ends of the tip and CE column were carefully polished flat with a scribe as observed under a microscope. A liquid–metal junction for the electrospray interface was created with a polyether ether ketone (PEEK) Micro Tee. To minimize postcolumn peak broadening caused by the dead volume (∼29 nL) of the small-diameter channel of the tee, it was enlarged to 0.015 in. (1 in. = 2.54 cm). This diameter corresponds to the outer diameter of

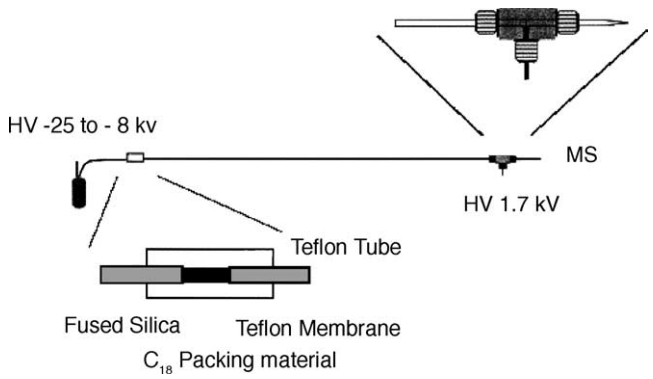

Fig. 5. Schematic of micro-tee CE–ESI-MS interface (from Ref. [34] with permission).

the CE capillary. The side channel of the tee was enlarged to 0.025 in. to accommodate a 1-in. piece of 0.025-in.-diameter gold wire. The electrospray tip and the separation capillary were carefully butted together along the channel of the Micro Tee and connected with fingertight microfittings. The 1-in. piece of 0.025-in.-diameter gold wire was inserted into the side channel of the Micro Tee to supply the electrical connection for the electrospray voltage, Fig. 5. The authors recommended, and rightfully so, that the butt ends of the capillary be flat and clean at the connection to minimize dead volume and misalignment at the junction and to ensure the efficiency of separation and transfer to the microspray tip.

Although these two-piece designs produce workable interfaces they may suffer from misalignment and imperfect butting of the two pieces of capillaries that result in sample loss and broadened separation zones.

4.3. Three-piece design

A three-piece design whereby electrical contact is established through a porous glass joint that is butt connected using PTFE sleeves, to the CE column from one side and the spray tip from the other has also been reported. Settlage et al. [35] explored the use of a porous glass joint, similar to that described above using fused silica capillary. Briefly, the polyimide layer was burned off a 2–3 mm piece of a 3 cm fused silica capillary and the exposed portion was etched with HF until the capillary wall was less than 20 μm. The entire porous glass joint was inserted into a piece of PTFE with a small notch cut through the center of the PTFE sleeve whose i.d. was matched to that of the capillary. The porous glass joint was then butt connected with the aid of a PTFE sleeve to the CE column, with the μESI emitter butt connected to the other side of the porous glass joint (Fig. 6). For on-line CE separations the porous fused silica was immersed in 1% acetic acid and the CE termination/μESI voltage of ca. +1.5 kV was applied to the acid solution. The μESI tip was positioned 1–2 mm from the heated capillary orifice to the MS system.

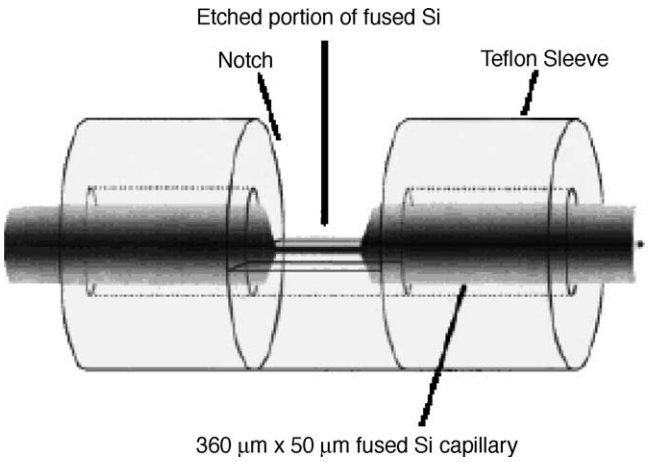

Fig. 6. Porous glass joint: center of fused silica capillary is etched so that the capillary walls are less than 20 μm thick and is centered in notch of PTFE sleeve (from Ref. [35] with permission).

An excellent and comprehensive review on recent advances in CE–ESI-MS was recently published [36].

5. Conclusions

Although the two- and three-piece designs produce operational interfaces, their fabrication requires delicate manipulation of miniaturized components and they suffer in their robustness and durability. In the two-piece approach, the CE-separated zones are invariably broadened at the junction between the separation column and the tip, since the inside diameter of the sleeve has to be larger than the outside diameter of the separation capillary. Furthermore, these junctions could suffer from misalignment and imperfect butting of the two pieces of capillary. Single-capillary methods appear to disrupt the CE separation the least; however, metal coatings on fused-silica capillaries are not durable and drilling pinholes through capillary walls is a delicate and irreproducible procedure. Once operational, a split-flow interface is highly sensitive, as demonstrated by Moini et al. [20], who reported the separation and detection of proteins from human red blood cells at attomole levels. The sheathless-single-capillary design presented by Janini et al. [25,26] seems to be simple, easy to fabricate, rugged and is not disruptive of the CE separation process.

Acknowledgements

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organization imply endorsement by the US Government. This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. NO1-CO-12400.

References

[1] T.J. Thompson, F. Foret, P. Vouros, B.L. Karger, Anal. Chem. 65 (1993) 900.
[2] J.A. Olivares, N.T. Nguyen, C.R. Yonker, R.D. Smith, Anal. Chem. 59 (1987) 1230.
[3] R.D. Smith, C.J. Barinaga, H.R. Udseth, Anal. Chem. 60 (1988) 1948.
[4] E.D. Lee, W. Muck, J.D. Henion, T.R. Covey, Biomed. Environ. Mass Spectrom. 18 (1989) 844.
[5] J.H. Wahl, D.C. Gale, R.D. Smith, J. Chromatogr. A 659 (1994) 217.
[6] J.F. Kelly, L. Ramaley, P. Thibault, Anal. Chem. 69 (1997) 51.
[7] J. Ding, P. Vouros, Anal. Chem. 71 (1999).
[8] J. Cai, J. Henion, J. Chromatogr. A 703 (1995) 667.
[9] M.B. Barraso, A.P. de Jong, J. Am. Soc. Mass Spectrom. 10 (1999) 1271.
[10] J. Samskok, M. Wetterhall, S. Jacobsson, K.J. Markides, Mass Spectrom. 35 (2000) 919.
[11] Y.Z. Chang, G.R. Her, Anal. Chem. 72 (2000) 626.
[12] D. Figeys, I. Oostveen, A. Ducert, R. Aebersold, Anal. Chem. 68 (1996) 1822.
[13] M.S. Kriger, K.D. Cook, R.S. Ramsey, Anal. Chem. 67 (1995) 385.
[14] M. Wilm, M. Mann, Anal. Chem. 68 (1996) 1.
[15] E.P. Maziarz, S.A. Lorentz, T.P. White, T.D. Wood, J. Am. Soc. Mass Spectrom. 11 (2000) 659.
[16] L. Fang, R. Zhang, E.R. Williams, R.N. Zare, Anal. Chem. 66 (1994) 3696.
[17] P. Cao, M. Moini, J. Am. Soc. Mass Spectrom. 8 (1997) 561.
[18] Y.Z. Chang, G.R. Her, Anal. Chem. 23 (2000) 626.
[19] M. Moini, Anal. Chem. 73 (2001) 3497.
[20] M. Moini, S.M. Demars, H. Huang, Anal. Chem. 74 (2002) 3772.
[21] M.A. Petersson, G. Hulthe, E. Fogelqvist, J. Chromatogr. A 854 (1999) 141.
[22] M. Mazereeuw, A.J.P. Hofte, U.R. Tjaden, J. van der Greef, Rapid Commun. Mass Spectrom. 11 (1997) 981.
[23] G.A. Valaskovic, F.W. McLafferty, J. Am. Soc. Mass Spectrom. 7 (1996) 1270.
[24] R.S. Ramsey, S.A. McLuckey, J. Microcol. Sep. 7 (1995) 461.
[25] G.M. Janini, T.P. Conrads, K.L. Wilkens, H.J. Issaq, T.D. Veenstra, Anal. Chem. 76 (2003) 1615.
[26] G.M. Janini, M. Zhou, L.R. Yu, J. Blonder, M. Gignac, T.P. Conrads, H.J. Issaq, T.D. Veenstra, Anal. Chem. 75 (2003) 5904.
[27] K.P. Bateman, R.L. White, P. Thibault, Rapid Commun. Mass Spectrom. 11 (1997) 307.
[28] J.T. Wu, M.G. Qian, M.X. Li, D.M. Lubman, Anal. Chem. 68 (1996) 3388.
[29] M.E. McComb, A.N. Krutchinsky, W. Ens, K.G. Standing, H. Perreault, J. Chromatogr. A 800 (1998) 1.
[30] D.R. Barnidge, S. Nilsson, K.E. Markides, H. Rapp, K. Hjort, Rapid Commun. Mass Spectrom. 13 (1999) 994.
[31] J.H. Wahl, R.D. Smith, J. Cap. Electrophor. 1 (1994) 62.
[32] J.C. Severs, R.D. Smith, Anal. Chem. 69 (1997) 2154.
[33] D. Figeys, A. Ducret, J.R. Yates, R. Aebersold, Nat. Biotechnol. 14 (1996) 1579.
[34] W. Tong, A. Link, J.K. Eng, J.R. Yates, Anal. Chem. 71 (1999) 227035.
[35] R.E. Settlage, P.S. Russo, J. Shabanowitz, D.F. Hunt, J. Microcolumn Sep. 10 (1998) 28136.
[36] A. Von Brocke, G. Nicholson, E. Bayer, Electrophoresis 22 (2001) 1251.