Compatibility of Agilent Jet Stream thermal gradient focusing technology with CE/MS

Technical Overview

Authors

Isabelle Kohler, Julie Schappler,
Serge Rudaz
School of Pharmaceutical Sciences
University of Geneva
Geneva, Switzerland

Hans-Peter Zimmermann,
Christian Wenz
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

Coupling capillary electrophoresis to mass spectrometers through the Agilent triple tube coaxial sheath-flow sprayer is a widely used configuration due to its simplicity, versatility, and robustness. This Technical Overview shows that with the Jet Stream source and the latest version of the CE/MS sprayer, this approach can now also be used in combination with the proprietary Agilent Jet Stream thermal gradient focusing technology. The sprayer worked as well on a mass spectrometer equipped with a conventional ESI source and showed, in comparison to the standard sprayer, a slightly increased performance. When the sprayer was used in combination with an Agilent Jet Stream source, equivalent or better results were obtained as compared to a conventional ESI source.

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Introduction

Achieving improvements in sensitivity and efficiency for electrospray ionization (ESI) has been a challenge over the past few decades, however, these improvements are important for multiple analytical applications. One recent innovation was the introduction of the thermal gradient focusing technology that was implemented in the Agilent Jet Stream source[1,2]. In this design, a superheated nitrogen sheath gas confines the nebulizer spray to more efficiently dry ions and concentrates them in front of the sampling orifice of the mass spectrometer. Due to improved ion generation and sampling efficiency, a 5 to 10-fold improved sensitivity can be obtained in comparison to conventional ESI in liquid chromatography coupled with mass spectrometric detection (LC/MS). The Agilent Jet Stream source is the standard source that is delivered with all Agilent mass spectrometry systems.

This Technical Overview demonstrates the compatibility of the Agilent Jet Stream thermal gradient focusing technology with capillary electrophoresis coupled with mass spectrometric detection (CE/MS). For use with the Agilent Jet Stream source, an updated triple tube coaxial sheath-flow CE/MS sprayer (p/n G1607B) is available.

The first part of this Technical Overview shows that the Agilent Jet Stream-compatible CE/MS sprayer can be used on a mass spectrometer equipped with a conventional ESI source without loss of sensitivity, compared to the standard sprayer. The second part compares the performance of the Agilent Jet Stream-compatible CE/MS sprayer on a MS system equipped with an Agilent Jet Stream source and a conventional ESI source, respectively. An equivalent or better sensitivity was obtained with the Agilent Jet Stream source.

Experimental

All CE separations were performed on an Agilent 7100 CE system. An Agilent 1260 Infinity Isocratic Pump equipped with degasser and 1:100 splitter was used for sheath liquid delivery. For CE and MS instrument control, Agilent ChemStation (revision B.04.03) and Agilent MassHunter (revision B.02.01, B2116.20, patches 1,2) installed on a single PC were employed. Prior to the actual measurements, optimal ion source parameters were determined by sample infusion. All measurement were repeated four times (n = 4).

Analysis of metamphetamine and methadone

Standard stock solutions in methanol of metamphetamine (MA), methadone (MTD) and their respective isotopically labeled derivatives at a concentration of 1,000 ppm were obtained from Lipomed (Arlesheim, Switzerland). Samples were prepared daily by diluting stock solutions in water.

CE conditions

Capillary:	Fused-silica capillary (BGB Analytik AG, Böckten, Switzerland), 50 µm id., total length 80 cm
Capillary coating:	CEofix dynamic coating compatible with MS detection (Analis, Namur, Belgium)
Background electrolyte (BGE):	25 mM ammonium acetate buffer, pH 5.0
Voltage:	+30 kV with a 0.3 minute voltage ramp
Temperature:	25 °C
Daily conditioning:	Flush (2 bars) with methanol for 5 minutes, water for 5 minutes, CEofix initiator and CEofix accelerator for 0.4 minutes each and BGE for 5 minutes
Pre-run conditioning:	Flush (2 bars) with BGE for 3 minutes
Post-run conditioning:	Flush (2 bars) with CEofix accelerator 0.5 minutes
Injection:	Sample, 50 mbar for 10 seconds and BGE, 50 mbar for 2 seconds

MS conditions

Instrument:	Agilent 6210 TOF
CE/MS sprayer version:	G1607A or G1607B
Ion source:	Dual ESI
Polarity:	ESI-positive
Fragmentor voltage:	150 V
Nebulizer gas pressure:	4 psi
Drying gas flow rate:	4 L/min
Drying gas temperature:	250 °C
Capillary voltage:	4,500 V
Sheath liquid:	Isopropanol-water-formic acid (50:50:0.5, v/v/v)
Sheath liquid flow rate:	3 µL/min
Scan rate:	2.5 spectra/s

Amino acid analysis

Samples were prepared daily by diluting an amino acid standard (1 nmol/µL, p/n 5061-3330) in water.

CE conditions

Capillary:	Bare-fused silica capillary, 50 µm id. total length 100 cm
BGE:	1 M formic acid
Voltage:	+30 kV with a 0.3-minute voltage ramp
Temperature:	20 °C
Pre-run conditioning:	Flush (1 bar) with BGE for 5 minutes
Injection:	Sample, 50 mbar for 8 seconds and BGE, 50 mbar for 2 seconds

MS conditions

Instrument:	Agilent 6320 TOF
CE/MS sprayer version:	G1607B
Ion source:	Agilent Jet Stream or Dual ESI
Polarity:	ESI-positive
Fragmentor voltage:	120 V
Nebulizer gas pressure:	10 psi
Drying gas temperature:	300 °C
Sheath liquid:	5 mM ammonium acetate in 50% methanol
Sheath liquid flow rate:	8 µL/min
Scan rate:	2 spectra/s

MS parameters for Agilent Jet Stream source only

Capillary voltage:	2,000 V
Nozzle voltage:	2,000 V
Drying gas flow rate:	8 L/min
Sheath gas temperature:	195 °C
Sheath gas flow rate:	3.5 L/min

MS parameters for Dual ESI source only

Capillary voltage:	4,000 V
Drying gas flow rate:	10 L/min

Organic acid analysis

Samples were prepared daily by diluting the organic acid test mixture (1,000 ppm, p/n 8500-6900) in water.

CE conditions

Capillary:	Bare-fused silica capillary, 50 µm id. total length 100 cm
BGE:	20 mM ammonium formate, pH 10
Voltage:	+30 kV with a 0.3-minute voltage ramp
Temperature:	20 °C
Pre-run conditioning:	Flush (1 bar) with BGE for 5 minutes
Injection:	Sample, 50 mbar for 8 seconds and BGE, 50 mbar for 2 seconds

MS conditions

Instrument:	Agilent 6320 TOF
CE/MS sprayer version:	G1607B
Ion source:	Agilent Jet Stream or Dual ESI
Polarity:	ESI-negative
Fragmentor voltage:	100 V
Nebulizer gas pressure:	10 psi
Drying gas flow rate:	10 L/min
Drying gas temperature:	300 °C
Sheath liquid:	5 mM ammonium hydroxide in 50% methanol
Sheath liquid flow rate:	8 µL/min
Scan rate:	2 spectra/s

MS parameters for Agilent Jet Stream source only

Capillary voltage:	1,500 V
Nozzle voltage:	2,000 V
Sheath gas temperature:	195 °C
Sheath gas flow rate:	3.5 L/min

MS parameters for Dual ESI source only

Capillary voltage:	4,000 V

Results and discussion

Comparing the Agilent Jet Stream-compatible sprayer and the standard sprayer

The use of the Agilent Jet Stream source for CE/MS is only possible with a dedicated version of the CE/MS sprayer. This Agilent Jet Stream-compatible sprayer has a modified tip and needle design and can be visually distinguished from the standard sprayer in a straightforward manner (Figure 1). The Agilent Jet Stream-compatible sprayer can also be used in combination with a conventional ESI source. The performance of this sprayer in comparison to the standard CE/MS sprayer was tested with a toxicological sample on a TOF/MS system equipped with a Dual ESI source (Figure 2). Peak areas obtained for all compounds were up to two-fold higher with the Agilent Jet Stream-compatible sprayer over the whole concentration range tested. Signal-to-noise ratios confirmed this tendency (data not shown). Peak area repeatabilities were for all compounds 10% RSD or lower. Therefore, the Agilent Jet Stream-compatible sprayer in combination with a conventional ion source shows a slightly better performance than the standard sprayer. One reason for this better performance is the improved mechanical design of the Agilent Jet Stream-compatible sprayer that helps to position the sprayer needle exactly in the center of the sprayer body. This should, in turn, improve spray quality and therefore ionization and signal intensity.

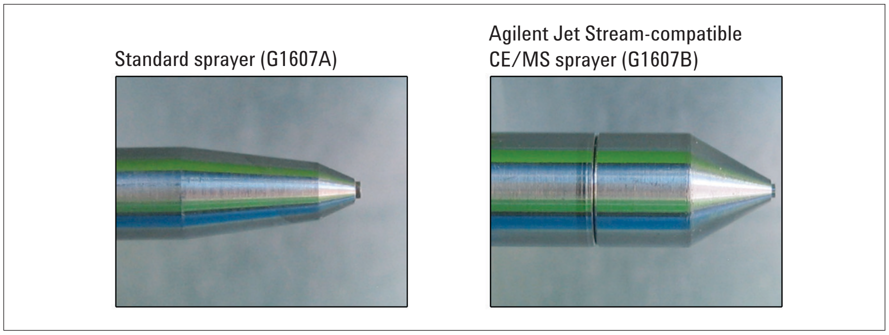

Figure 1
Photographs of the tips of different CE/MS sprayer versions.

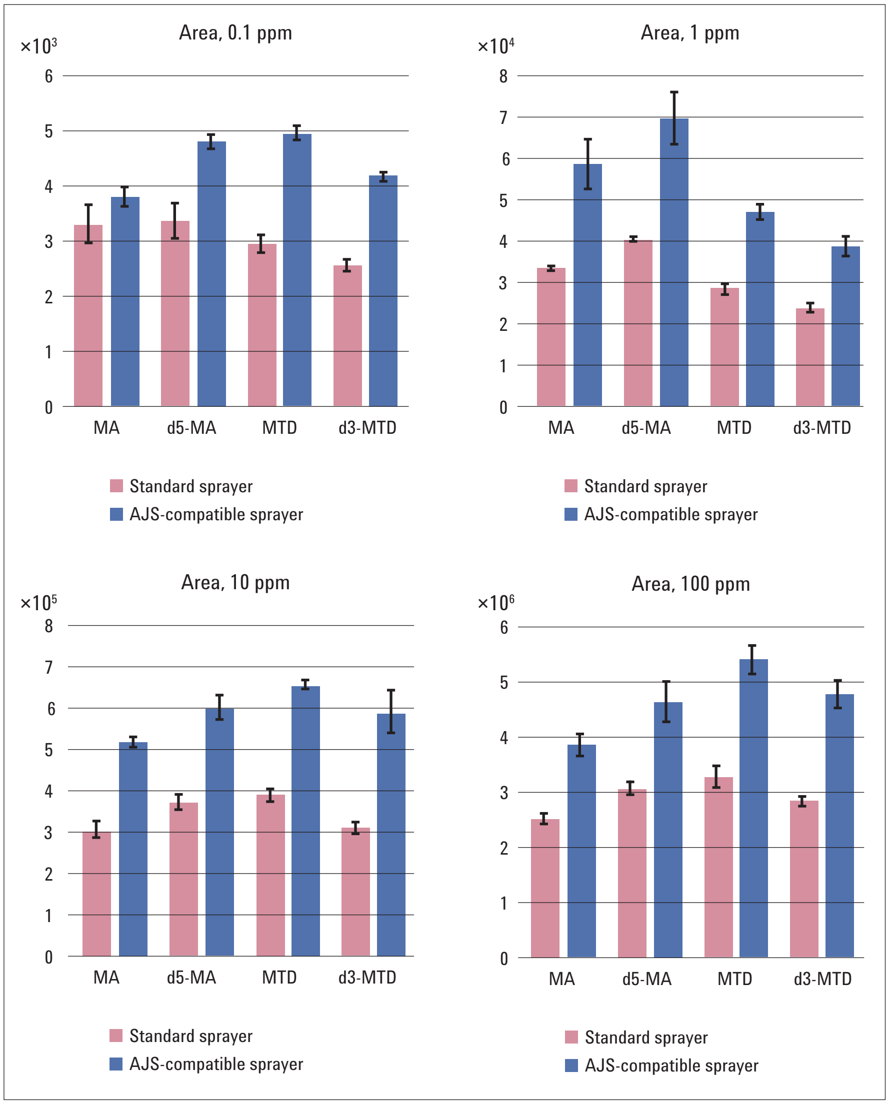

Figure 2
Comparison of standard and Agilent Jet Stream-compatible CE/MS sprayers on an MS instrument equipped with a Dual ESI source. Average peak areas (n = 4) with standard deviations obtained from extracted ion electropherograms of methadone, metamphetamine, and isotopically labeled derivatives at the indicated concentrations are shown. MA, Metamphetamine; d5-MA, d5-Metamphetamine; MTD, Methadone; d3-MTD, d3-Methadone.

Performance of the Agilent Jet Stream-compatible CE/MS sprayer in combination with different ion sources

A direct comparison of the performance of the Agilent Jet Stream-compatible sprayer on an MS instrument equipped with either an Agilent Jet Stream source or a conventional ESI source was done with small molecule standards in the positive and the negative ion mode. Figure 3 shows results obtained with an amino acid sample in the positive ion mode. On average, peak areas were slightly increased with the Agilent Jet Stream source. Peak area repeatabilities were 10% RSD or lower. With both ion sources, no background noise was observed for any amino acid except valine. To get an estimate of the sensitivity, different amino acid standard dilutions were analyzed down to a concentration of 0.01 µM. A similar number of detectable amino acids at given concentrations and therefore a similar sensitivity was observed with both ion source types (Figure 4). An at least equivalent sensitivity of the Agilent Jet Stream source in comparison to a conventional ESI source was obtained with sheath liquid flow rates in the range of 4–10 µL/min (data not shown).

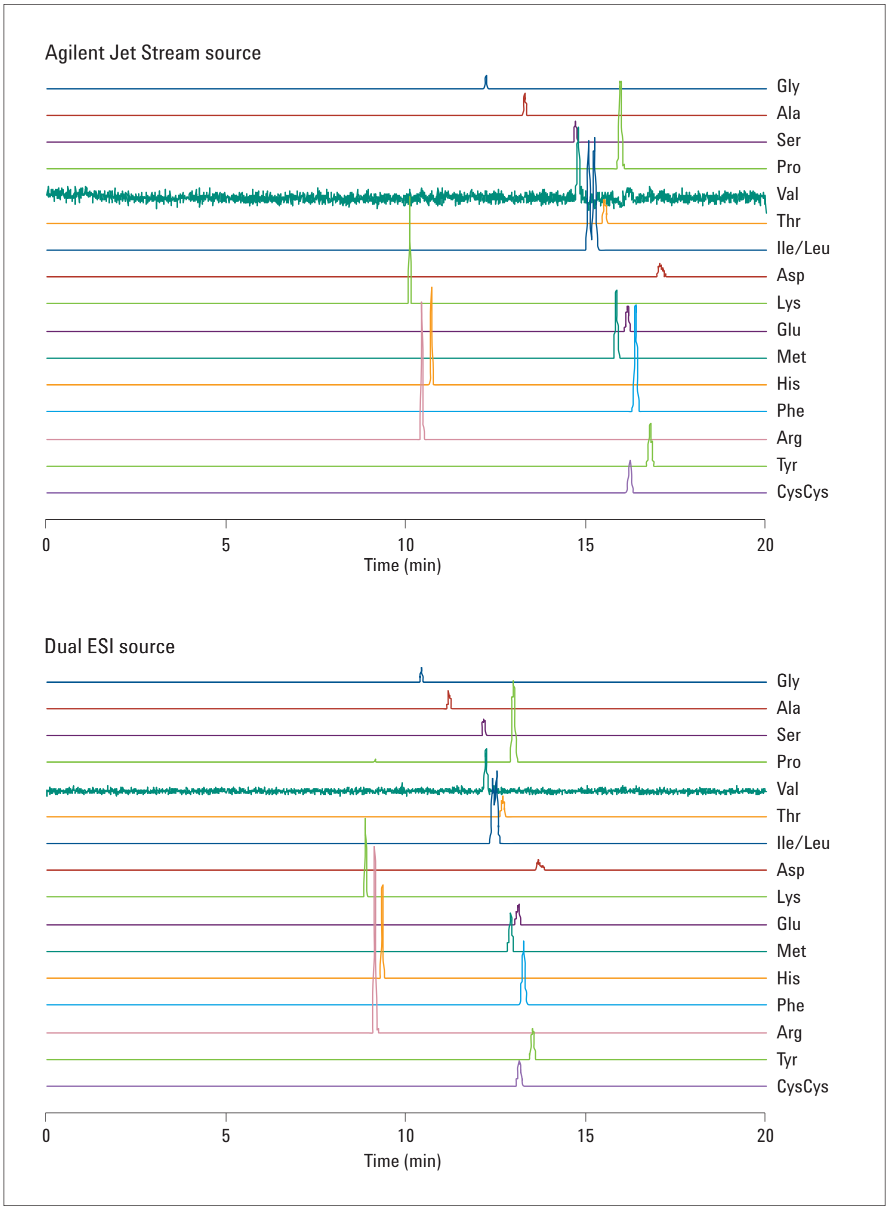

Figure 3
Performance of the Agilent Jet Stream-compatible CE/MS sprayer with different ion sources in the positive ion mode. Extracted ion electropherograms for all 17 amino acids present in the sample at a concentration of 10 µM are shown.

In summary, these data indicate a very comparable performance of the Agilent Jet Stream-compatible sprayer in combination with the Agilent Jet Stream source, versus the conventional Dual ESI sources in the positive ion mode. Results obtained with an organic acid sample in the negative ion mode are shown in Figure 5 and Table 1. In contrast to the results obtained in the positive ion mode, peak areas for all three sample compounds were 6–8 fold enhanced with the Agilent Jet Stream source. However, the signal-to-noise (s/n) was only similar or up to 4-fold increased due to the lower noise that was obtained with the Dual ESI source (Table 1). The performance of the Agilent Jet Stream compatible sprayer in combination with Agilent Jet Stream source in the negative ion mode was therefore improved, in comparison to the Dual ESI source for two out of three compounds tested.

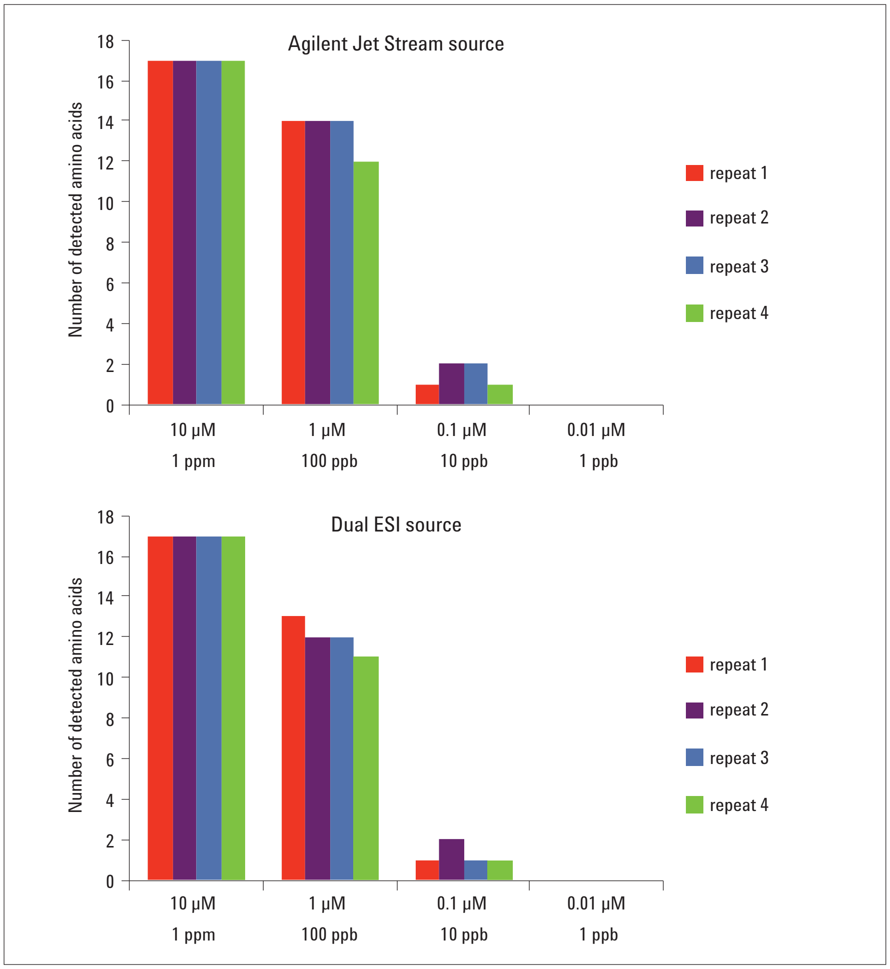

Figure 4

Limits of detection in the positive ion mode. Amino acid standard dilutions with concentrations of 10, 1, 0.1, and 0.01 µM were analyzed with different ion sources. The number of amino acids that could still be detected in the extracted ion electropherograms with a signal-to-noise ≥ 3 is shown. Concentrations in ppm/ppb units were calculated with a molecular weight of 100 g/mol.

Dual ESI source				
Analyte	**Area x 10^3**	**Area RSD%**	**s/n**	**s/n RSD%**
Lactic acid	7.0	3.1	3.5	9.4
Succinic acid	5.9	17	4.5	20
Malic acid	12	5.8	7.2	24
Agilent Jet Stream source				
Analyte	**Area x 10^3**	**Area RSD%**	**s/n**	**s/n RSD%**
Lactic acid	33	16	3.3	15
Succinic acid	49	8.8	8.4	17
Malic acid	77	6.3	26	12

Table 1

Results of the organic acid analysis in the negative ion mode. Shown are average peak areas and s/n with the corresponding repeatabilities (n=4) with different ion sources. Data were calculated from extracted ion electropherograms obtained at a sample concentration of 1 ppm. Peak-to-peak noise values were calculated over a 5 min interval in the vicinity of the peak of interest.

Sensitivity gains that can be obtained with the Agilent Jet Stream thermal gradient focusing technology in LC/MS are primarily due to more efficient ion drying and concentration by the superheated nitrogen sheath gas. However, these effects are flow rate dependent: a 5–10 fold increased sensitivity was seen with the Agilent Jet Stream source at flow rates ranging from 50 µL/min to 2.5 mL/min, with greatest gains typically at 0.25 to 1.0 mL/min[2]. The use of the Agilent Jet Stream source in combination with a dedicated microflow LC/MS sprayer enabled the extension of this range, sensitivity gains of 3–4 fold were reported for flow rates down to 15 µL/min[3,4]. However, typical flow rates for CE/MS employing the Agilent triple tube coaxial sheath flow interface are in the range of 1–10 µL/min. With the flow rate of 8 µL/min used, a substantial sensitivity gain was observed for only a fraction of the analytes tested in the negative ion mode, but not in the positive ion mode.

Furthermore, the best Agilent Jet Stream source parameters for CE/MS were found to be 195 °C and 3.5 L/min for sheath gas temperature and flow rate, respectively (cf. Experimental). These settings differ substantially from the standard values of 350 °C and 11 L/min that are recommended for best Agilent Jet Stream source performance in LC/MS[2]. These results may indicate that ion drying is not as critical for flow rates lower than 15 µL/min as compared to higher flow rates. Alternatively, principle differences in the design of LC/MS and CE/MS interfaces, for example, different sprayer tip dimensions, could limit the benefits that can be achieved with the Agilent Jet Stream thermal gradient focusing technology in CE/MS as compared to LC/MS.

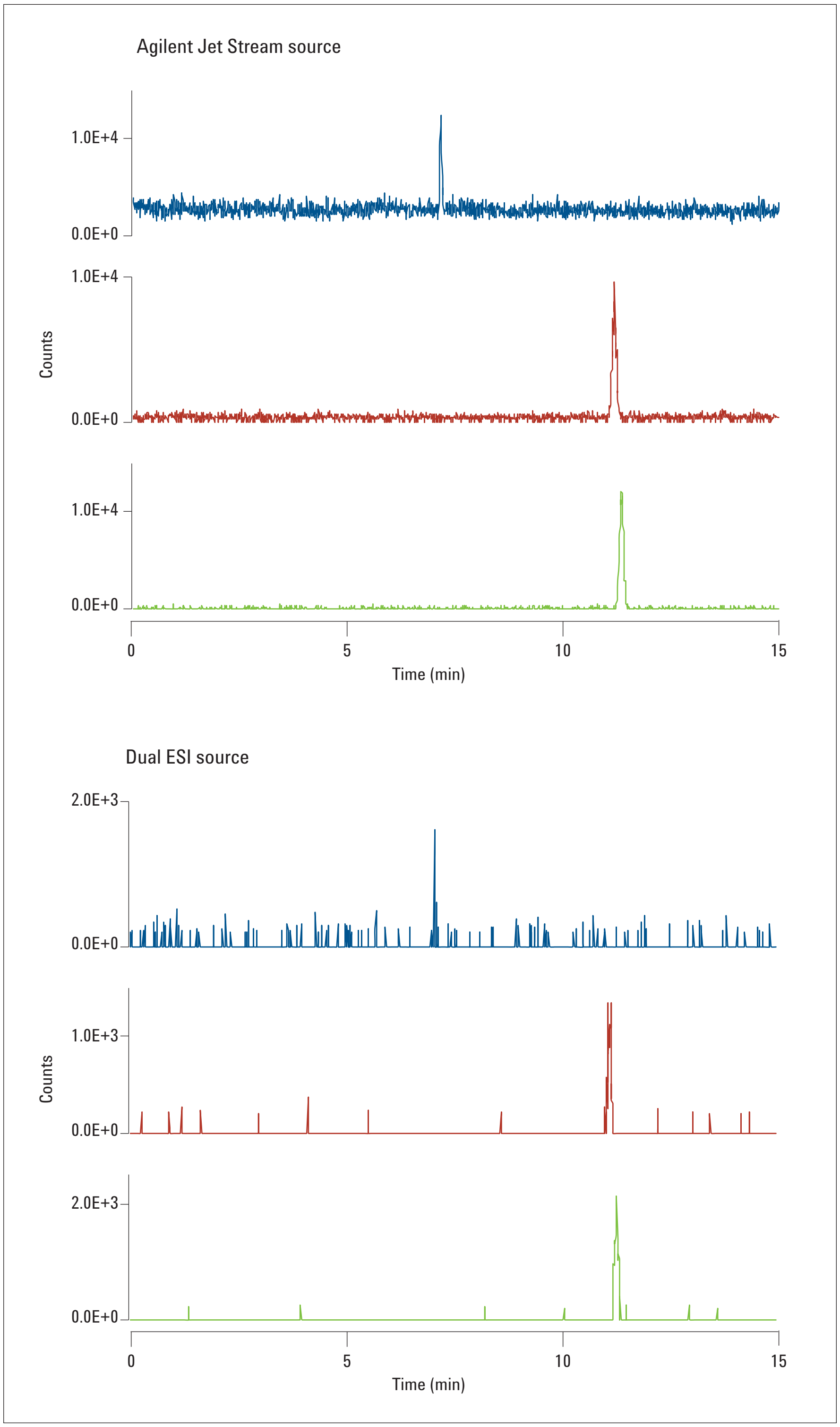

Figure 5

Performance of the Agilent Jet Stream-compatible CE/MS sprayer with different ion sources in the negative ion mode. Shown are extracted ion electropherograms for the three organic acids present in the sample at a concentration of 1 ppm (from top to bottom: lactic acid, succinic acid, and malic acid).

Conclusions

The Agilent Jet Stream thermal gradient focusing technology is compatible with CE/MS. The Agilent Jet Stream source can be used in combination with the G1607B version of the triple tube coaxial sheath-flow CE/MS sprayer. The Agilent Jet Stream-compatible sprayer fits as well into conventional ESI sources and showed a slightly improved performance as compared to the standard CE/MS sprayer. In a direct comparison of the performance of this sprayer on a MS instrument equipped with an Agilent Jet Stream source and a standard ESI source respectively, an at least equivalent or better sensitivity was obtained with the Agilent Jet Stream source.

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