Technical Overview

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Improved CE/MS Sensitivity by Operating the Triple-Tube Coaxial Sheath-Flow Sprayer Without Appling Nebulizing Gas

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Abstract

Traditionally, the Agilent triple-tube coaxial sheath-flow sprayer is operated with a nebulizing gas that helps establish the spray. Using a nebulizing gas has some disadvantages such as the so-called suction effect. Therefore, an investigation was performed to establish conditions that enabled operation of the triple-tube sprayer without nebulizing gas. A design-of-experiments approach was used to optimize the four most important parameters, that is, drying gas temperature, capillary voltage, sheath liquid flow rate, and capillary protrusion. High spray stability and excellent repeatability were observed under optimized conditions. The sensitivity was increased for most of the analyzed compounds when the triple-tube sprayer was operated without nebulizing gas in comparison to conventional conditions applying nebulizing gas.

Introduction

The Agilent triple-tube coaxial sheath-flow sprayer is a widely used interface for coupling capillary electrophoresis to mass spectrometers due to its simplicity, versatility, and robustness[1]. Traditionally, it is operated with a nebulizing gas delivered by the outer tube that helps in establishing the spray. However, applying a nebulizing gas has some disadvantages such as the slight suction it exerts on the liquid in the CE capillary. This suction creates a hydraulic flow towards the exit of the capillary (the so-called suction effect)[2,3]. The hydraulic flow through the capillary causes a parabolic solvent flow velocity profile, additional zone broadening, and a reduction of the efficiency of the analyte zones.

This Technical Overview presents a systematic investigation of conditions that enable the operation of the triple-tube sprayer without applying nebulizing gas. A design-of-experiments (DoE) approach was used to optimize the four most important parameters. The sensitivity of the optimized method was compared to conventional CE/MS conditions with nebulizing gas.

Experimental

Sample Solutions

Forty-six basic endogenous compounds and one basic polar drug (MDMA) were used as model compounds. Individual stock solutions were prepared in 5 % acetonitrile and 0.1 % formic acid (FA) except for neopterin and biopterin, which were prepared in DMSO and guanine in 1 M HCl. Mix stock solutions were prepared in 5 % acetonitrile and 0.1 % FA at 10 µg/mL, then diluted to 500 ng/mL using 50 mM FA.

Capillary Electrophoresis/Mass Spectrometry

Experiments were carried out with an Agilent 7100 capillary electrophoresis (CE) system from Agilent Technologies (Waldbronn, Germany). Separations were carried out using a fused silica capillary purchased from BGB technologies (Boeckten, Switzerland) with a 70 cm length and a 50 µm internal diameter. A new capillary was conditioned with the following solvents at 5 bar for one minute each in the following order:

1. MeOH
2. H_2O
3. 1 M NaOH
4. H_2O
5. 1 M HCl
6. H_2O
7. 0.1 M HCl
8. H_2O
9. BGE

Between each run, the capillary was rinsed using background electrolyte (BGE) at 5 bar for one minute. Hydrodynamic injections were performed (50 mbar × 12 seconds) corresponding to injected volumes of 13.7 nL (1 % of the capillary volume), calculated using Zeecalc v1.0 (http://www.unige.ch/sciences/pharm/fanal/lcap/zeecalc/zeecalc.zip). Separations were performed at 30 kV using 10 % acetic acid as the BGE. Samples were kept around 10 °C in the autosampler using an external water-cooling system purchased from VWR (Nyon, Switzerland) set to 1 °C.

The CE system was coupled with an Agilent 6490 triple quadrupole LC/MS purchased from Agilent Technologies (Santa Clara, CA, USA) equipped with an electrospray ionization (ESI) source through a coaxial sheath-flow ESI interface with a standard triple-tube sprayer (p/n G1607B) from Agilent Technologies (Waldbronn, Germany). For all experiments, EMV voltage, drying gas flow rate, high-pressure RF, and low-pressure RF were respectively set at 400 V, 11 L/min, 150 V, and 60 V. Data acquisition and instrument controls were monitored using Agilent MassHunter software version B.08.00 (Agilent, Santa Clara, CA, USA).

Results and Discussion

Source Parameter Optimization

Among the experimental parameters affecting the ionization process in CE/MS, the nebulizing gas appeared highly detrimental to peak intensity and efficiency. Therefore, it was decided to shut down the nebulizing gas, and optimize other parameters using a DoE approach. Four operational parameters were evaluated:

- Drying gas temperature (X_1)
- Capillary voltage (X_2)
- Sheath liquid flow rate (X_3)
- Capillary protrusion (X_4).

MDMA intensity was chosen as the response. A Box-Behnken design was selected; the experimental plan consisted of two variables set in a combination of their extreme values, while the other variable was set to the midpoint value. To estimate the method error, 24 experiments were performed in addition to three trials at the center of the investigated domain. Table 1 summarizes the investigated ranges for each factor. To ensure a measurable CE/MS signal, the assessment of low (–1) and high (+1) levels were determined during the qualification stage.

The DoE highlighted that models were significant for MDMA intensity with p-values lower than 0.1 %. Figure 1 presents the relationship between predicted and observed values. A statistical study of the coefficients revealed that the responses fit adequately, with a determination coefficient (R^2) higher than 89 %.

A broad repartition of the experimental points was observed, and three areas were emphasized:

- High responses (assays 7, 13, 14, 17, 20, 21, and 27)
- Medium responses (assays 10, 18, 19, 24, 25, and 26)
- Low responses (assays 1, 2, 3, 4, 5, 6, 8, 9, 11, 12, 15, 16, 22, and 23)

Analytical responses were on a 380-fold range, demonstrating that an important gain in sensitivity could be obtained by an appropriate setting of the four parameters. Figure 2 shows the effect of the parameters on the response with the response surface with gas temperature and sheath-liquid flow rate respectively set at 165 °C and 2 µL/min. The model highlighted the positive influence of the capillary protrusion and the capillary voltage on the response. The drying gas temperature and the sheath liquid flow rate appeared to be of lower influence. Based on these results, the following were predicted to be optimal conditions for MDMA intensity:

- No nebulizing gas
- Capillary voltage of 5,500 V
- Capillary protrusion of +3 (~8 × 10^{-3} in)
- Drying gas temperature of 200 °C
- Sheath liquid flow rate of 2 µL/min

Table 1. DoE Investigated parameters.

Level	X_1 Drying gas temperature (°C)	X_2 ESI Voltage (V)	X_3 Sheath liquid flow rate (µL/min)	X_4 Capillary protrusion (marks/×10^{-3} inch)
–1	80	4,000	2	0/0
0	165	5,000	6	+2/~6
1	250	6,000	10	+4/~11

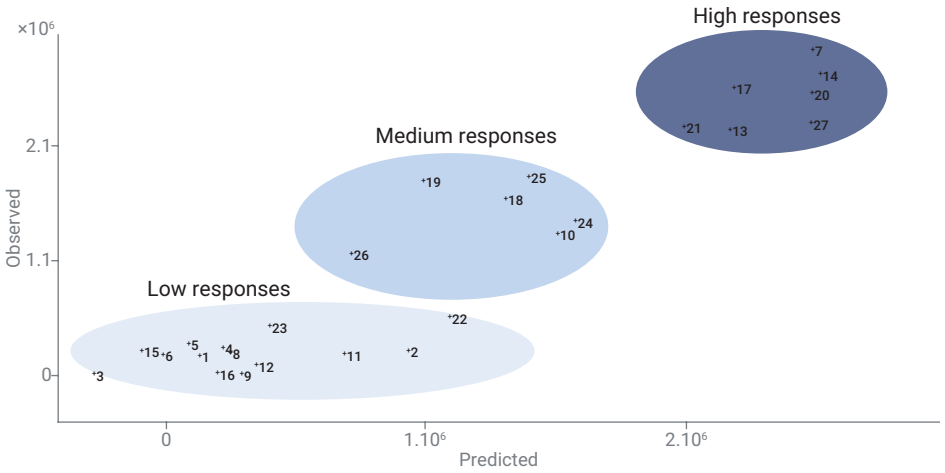

Figure 1. Optimization of the source parameters: observed versus predicted values of MDMA intensity.

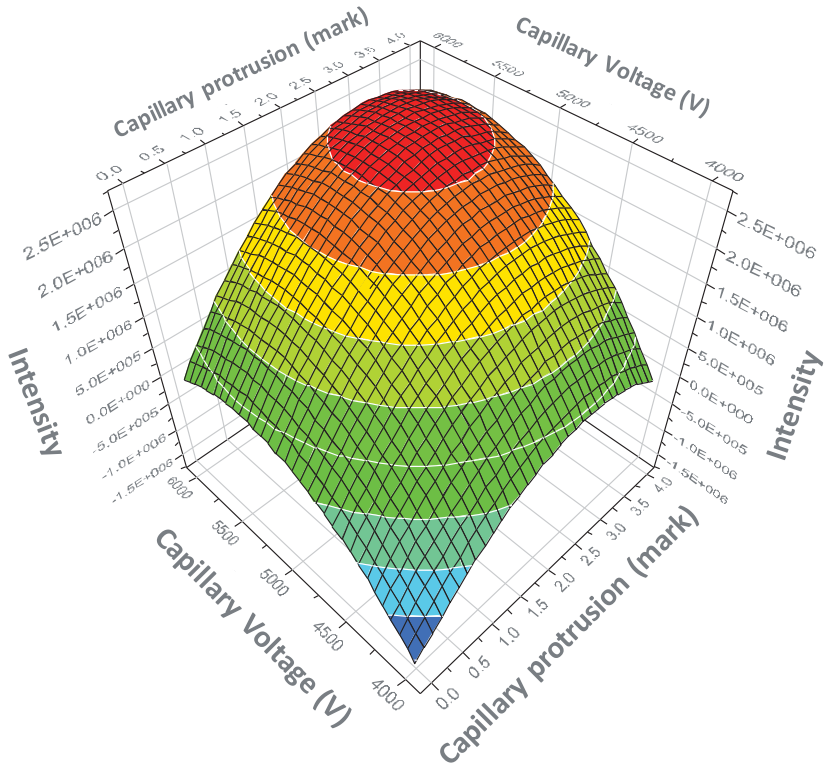

Figure 2. Response surface for MDMA intensity (gas temperature and sheath-liquid flow rate respectively set at 165 °C and 2 µL/min).

Conventional Conditions Versus Optimized Conditions

To analyze numerous basic endogenous compounds, optimized conditions were compared to conventional CE/MS conditions[4]:

- Nebulizing gas at 4 psi
- Capillary voltage of 4,500 V
- Capillary protrusion of +2 (~6×10^{-3} in)
- Drying gas temperature of 250 °C
- Sheath liquid flow rate of 3 µL/min

Figure 3 shows a gain in sensitivity for most of the analyzed compounds under optimized conditions without nebulizing gas. The signal-to-noise ratio (S/N) was not significantly changed for seven compounds (15 % of all compounds: $S/N_{without\ nebulizing\ gas}/SN_{conventional}$ between 0.5 and 1.5), 30 compounds showed a moderate increase (63 %; 1.5 to 4.5), and 11 compounds a substantial increase of

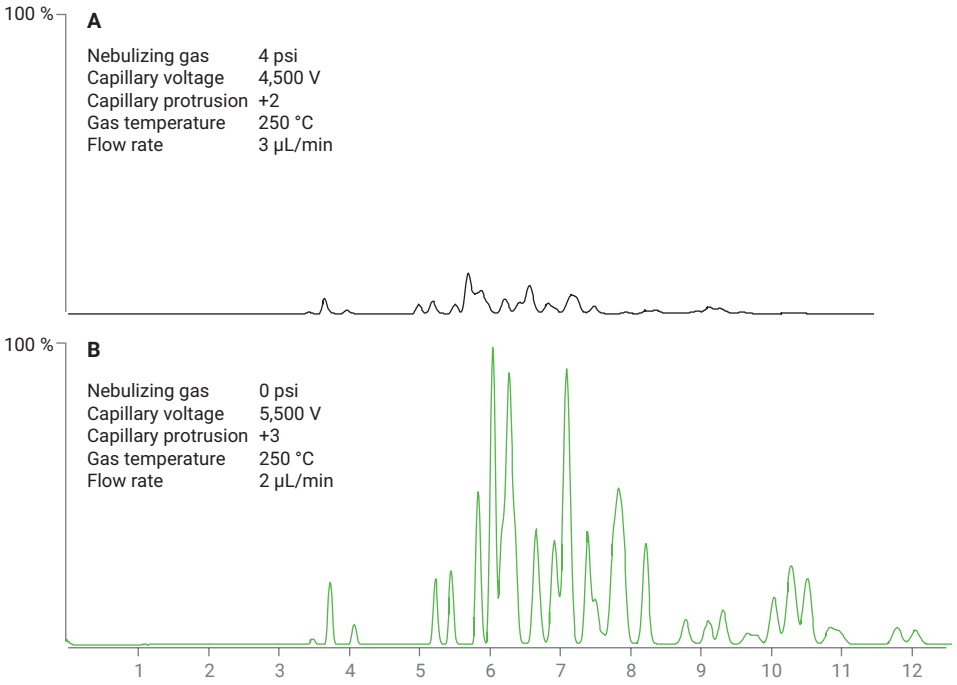

Figure 3A. Example electropherograms, shown as a comparison of conventional source parameters (A) versus optimized conditions without nebulizing gas (B).

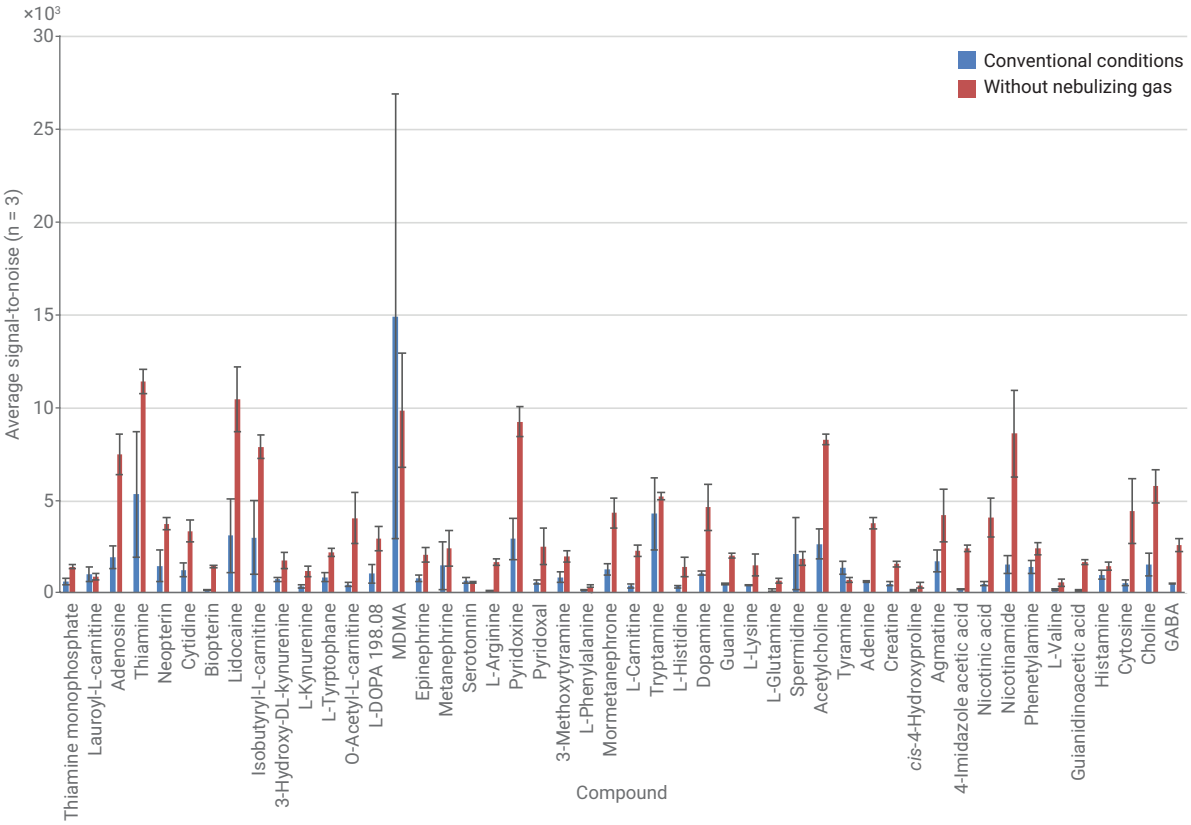

Figure 3B. Comparison of conventional source parameters versus optimized conditions without nebulizing gas. Shown is a plot of average S/N values with standard deviation for all analyzed compounds.

S/N under optimized conditions, without nebulizing gas (23 %, 4.5 to 11.5).

Because of the significant reduction of the suction effect due to the eliminated nebulizing gas, an increase in analysis time was observed. Consequently, efficiency and resolution were improved. The stability of the spray was controlled over 14 runs. Excellent repeatability was obtained, with area RSD for MDMA and L-arginine below 5 %, although no internal standard was used for area correction (Figure 4).

Conclusion

Using the conventional interface configuration, nebulizing gas appeared to be detrimental to sensitivity and resolution due to the suction effect. The nebulizing gas was switched off, involving the adjustment of other parameters (such as capillary protrusion and capillary voltage). Using the optimized source conditions, the sensitivity was improved for most of the analyzed compounds, keeping high spray stability, and excellent repeatability.

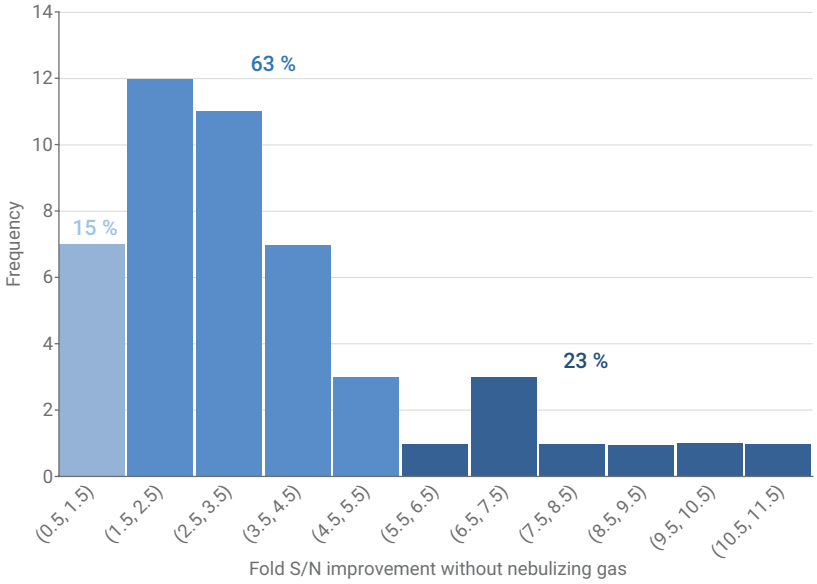

Figure 3C. Comparison of conventional source parameters versus optimized conditions without nebulizing gas. Shown is a histogram of S/N for all analyzed compounds.

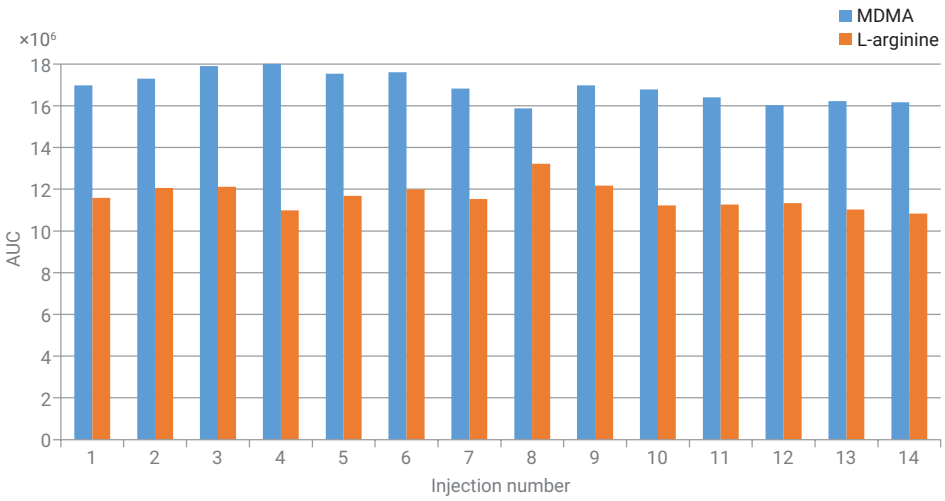

Figure 4. Comparison of MDMA and L-arginine area under the curve from 14 successive injections.

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