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SHORT COMMUNICATION

Enhanced Nebulization Efficiency of Electrospray Mass Spectrometry: Improved Sensitivity and Detection Limit

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Abstract

A novel electrospray nebulizer has been designed, which includes an additional nebulization gas capillary inside the liquid capillary. This design offers significantly enhanced ionization efficiency compared with the classic nebulizer design and leads to improved sensitivity (by three to 10 times) and decreases the detection limit, on an average 10 times. We see these results as the first step in the design of ESI nebulizers offering improved sensitivity and higher robustness. Possible future developments would include optimization of the dimensions of the capillaries as well as testing the nebulizer for other matrices and analytes.

Key words: LC/MS, ESI, Nebulizer, Pesticides

Introduction

Several new developments—an additional wire inside the liquid capillary [1], asymmetrically cut liquid capillary [2], etc.—in the nebulizer design have been proposed recently, aiming at improvement of sensitivity and robustness of ESI nebulization. Significant improvement of sensitivity has been shown by several authors at lower flow rates (micro- and nano-ESI sources as well as for flow-splitting) [3–5].

The downsides of nano-ESI are that it is not easily usable for coupling LC and MS [6], the conventional ESI needles may have nonoptimal dimensions for some lower flow rates [3], and nano-ESI is known to be significantly less robust than ESI [6]. Therefore, a nebulizer resulting in the same benefits as nano-ESI but working under ESI condition would be extremely beneficial.

In this report, we introduce a new ESI sprayer design, where an additional nebulizer gas capillary is added inside the liquid capillary. This article does not present a fully geometrically optimized source design, but rather a new electronebulization concept using a coaxial nebulizer gas.

Experimental

Ionization Source

The novel ESI nebulizer was constructed of three concentrically arranged stainless steel tubings with o.d. and i.d., respectively, 4 and 2 mm, 0.8 and 0.55 mm, and 0.203 and 0.089 mm [7]. In this paper, these tubings are called capillary A, capillary B and capillary C, respectively. Construction of the nebulizer allows easy adjustment of lengths of all the capillaries, but in present study lengths of capillaries A and B were fixed in order to limit the number of variables in the experimental design. The length of the capillary A was 53 mm and the capillary B was extending from it by approximately 1/3 of its o.d. (0.27 mm). The capillary C was removable and its position relative to the capillary B (i.e., the length of C reaching out of B) was changeable but was fixed to 0 mm in order to achieve

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better repeatability in positioning the capillary. Please see Supplementary Information for a graphical overview.

Two nebulizer designs were used:

1. Design 1 was a conventional design with two capillaries, A and B. The capillary B, where the liquid chromatography effluent flows, and around it the capillary A, where the nebulizer gas flows.
2. For Design 2, the capillary C was added to the system (inside capillary B) and connected to a nitrogen source and nitrogen flow was used for additional nebulizing effect.

The dimensions of the built nebulizer are somewhat different from the commercially available nebulizers. Finding the optimal diameters for all three capillaries will be left for future developments.

Chemicals, LC and MS Parameters

The information about the used chemicals, LC as well as MS parameters can be found in Supplementary Information or described by Kruve et al. [8].

Results and Discussion

Optimization

Optimization of both gas flow rates, in capillaries A and C, was carried out with infusion experiments. The optimization plot of the gas flow rate in capillary C (Design 2) is presented in Figure 1. All other optimization plots are presented in Supplementary Information.

During capillary C gas flow optimization (Design 2) the nebulizer gas flow rate in capillary A was fixed to 19 L/min (a medium possible flowrate). For capillary C it was observed (Figure 1) that independent of the eluent composition (%MeOH), the MS signal significantly increases with increasing gas flow rate, meaning that the nebulizer gas from capillary C significantly improves the nebulization and increases the MS signal. The maximum possible flow rate 300 mL/min from capillary C gave 2.7 to 17.2 times higher sensitivity depending on the eluent composition compared to 0 mL/min.

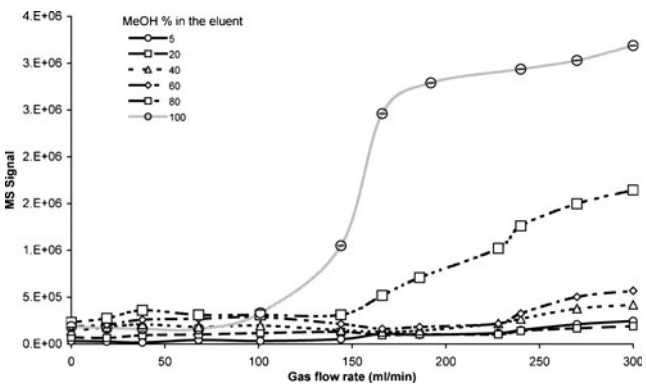

Figure 1. The MS signal intensity dependence on the gas flow rate in capillary C at different effluent compositions. Each point (intersection of two lines) represents the average of three measurements, error bars are not indicated as these are smaller then the point and the points are connected with splines

For capillary A the optimization plots were markedly different for Designs 1 and 2. For Design 1, the optimal flow rate was 28.5 L/min, the highest flow rate possible because of the instrumental limitations. For Design 2, the optimal flow rate was 3.8 L/min, the minimum flow rate allowed by the software. This demonstrates that even though the dimensions of the capillaries in the nebulizer are not optimized, the inner capillary C does lead to improved nebulization. This result envisages a reversed ESI nebulizer design, whereby the nebulization gas is blown only inside the liquid stream.

Sensitivity

The sensitivities of the two nebulizer designs were compared according to calibration graph slopes (linear regression from 0.1 to 1.0 or 5.0 mg/kg depending on the analyte and matrix) in solvent, onion, and garlic with optimized ESI/MS parameters. For all but one calibration graph, nebulizer Design 2 with the capillary C resulted in higher sensitivity. The slopes were 3.1 (for imazalil in garlic) to 10.2 (for methiocarb in solvent) times higher for design 2 (capillary C gas 300 mL/min and capillary A gas 3.8 L/min) compared with the nebulizer Design 1 without capillary C. As an example, the calibration graphs for thiabendazole in garlic are presented in Figure 2. Other graphs are shown in the Supplementary Information. These results indicate that nebulizer Design 2 results is more effective nebulization and most probably also smaller droplets. Differently from

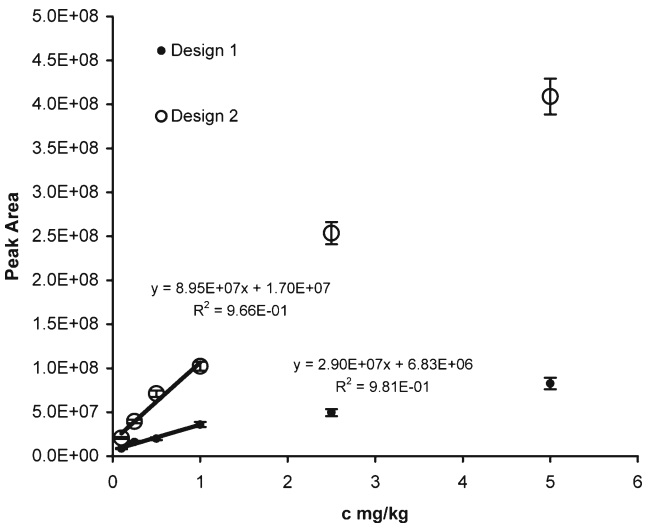

Figure 2. Thiabendazole calibration graphs in spiked garlic extracts with different nebulizer designs

other pesticide-matrix combinations for methiocarb in garlic, nebulizer Design 2 gave lower responses compared with Design 1. Garlic is known to be the worst case matrix for pesticide analysis [9]. More effective nebulization achieved with nebulizer Design 2 is assumed to reduce competition for the surface of the droplet and, therefore, we assume that a different type of matrix effect occurs for methiocarb in garlic analysis (for example gas-phase proton transfer).

From these results, it can be concluded that the additional nebulizer capillary helps to improve the sensitivity both in solvent as well as in complicated matrix extracts.

Limit of Detection

The LoD values were found for the two sets of operation conditions in solvent, apple extract and garlic extract. The solutions with concentrations of 50, 25, 10, 5, 2.5, 1.0, 0.5, 0.2, and 0.1 μg/kg were prepared and injected in a random order. For each analyte-matrix combination the lowest concentration resulting in a peak with signal-to-noise ratio (RMS noise) of at least 3 was assigned as LoD. Signal-to-noise ratios were obtained with Data Analysis software ver. 5.2, which calculates noise over the whole chromatogram except the peaks. The corresponding chromatograms are presented in the Supplementary Information and the found LoD values are presented in Table 1. The LoD for carbendazim in apple matrix could not be determined due to the fact that the apple extract already contained carbendazim. In some cases, the signal-to-noise ratio in the solution of the lowest concentration where the analyte could still be detected was significantly above 3. In those cases, a conservative approach was taken: no attempt was made to extrapolate the LoD to a lower value (with signal-to-noise ratio corresponding to 3). Instead, this lowest concentration, where the analyte was actually detected, was taken as an estimate of LoD. This approach is especially relevant in our case because the dependence of S/N ratio on concentration is strongly nonlinear. This can be one of the reasons for the seemingly high LoD-s observed in some cases for solvent analysis compared to the sample analysis.

An anomalously low LoD was observed for carbendazim in garlic extract (0.1 μg/kg) compared with the LoD in solvent (5.0 μg/kg). Neither carry-over, signal enhancement, nor false positive could be the reasons. The study of the chromatograms revealed that the noise level in the case of the standards analysis is somewhat higher near the carbendazim peak compared with the garlic samples analysis in the case of nebulizer Design 2. The LoD values were rechecked on a different day with similar results.

Table 1 shows that the LoD values for Design 2 are lower than for Design 1. The average improvement of LoD is 10 times (found as geometric mean of the pairwise ratios of LoD values), the largest improvement is 250 times (carbendazim in garlic). It was of interest to compare the obtained LoD values with those achievable with the native commercial nebulizer of the used MS system. It was found that Design 2 gives lower or comparable values. With the commercial nebulizer the LoD values were 1.0, 1.0, 1.0, and 5.0 μg/kg in solvent and 1.0, 1.0, 5.0, and >50.0 μg/kg in garlic for carbandazim, thiabendazole, imazalil, and methiocarb, respectively (obtained with MS parameter sets optimized for sensitivity as described by Kruve et al. [14]). Comparing these data to Table 1 indicates that the novel nebulizer gives on the average 1.7 times lower LoD values (found as geometric mean of the pairwise ratios of LoD values). This is a very good result, considering that the dimensions of the novel nebulizer are unoptimized at this stage. This implies that still significantly better performance in terms of sensitivity and LoD may be achieved with the newly designed nebulizer after optimizing the dimensions and positions of the capillaries.

Repeatability

The repeatabilities (RSD values over five replicates) were compared with F-test for solvent, apple, and garlic matrix. It was found that for all of the pesticides both nebulizer designs gave comparable repeatabilities—RSD ranging between 3 % and 8 %—with no statistically significant differences.

Robustness

Garlic matrix yields an extract with numerous compounds and tends to strongly contaminate the MS system leading to decreased or increased signals. In order to demonstrate the robustness of nebulizer Design 2 (i.e., its ability to avoid such contamination, 20 injections of the garlic extract spiked with pesticides at 0.5 mg/kg level were injected in a row. The graphical representation of the results is available in

Table 1. LoD Values Together with the S/N Ratios (in Brackets) for Two Different Nebulizer Designs. All Values are in μg/kg

	Design 1 (no capillary C and capillary A gas 28.5 L/min)			Design 2 (capillary C gas flow rate 300 mL/min capillary A gas 3.8 L/min)		
	Solvent	Apple extract	Garlic extract	Solvent	Apple extract	Garlic extract
Carbendazim	10.0 (21)	NA	25.0 (7.1)	5.0 (18)	NA	0.1 (7.3)
Thiabendazole	1.0 (9.2)	1.0 (12)	25.0 (17)	0.2 (8.9)	0.1 (5.9)	0.2 (6.3)
Imazalil	1.0 (6.9)	1.0 (16)	25.0 (6.9)	0.2 (8.0)	0.1 (7.9)	25.0 (22)
Methiocarb	50.0 (75)	50.0 (43)	>50.0	5.0 (7.9)	10.0 (5.8)	>50.0

Supplementary Information. The peak areas with nebulizer Design 2 were significantly more stable than with the commercial nebulizer. With commercial nebulizer the signal for imazalil decreased by as much as 57 % compared with the first injection, and a very sharp decrease in signal was observed between injections 6 and 9. On the other hand, for nebulizer Design 2, only a gentle decrease of signal intensity (max decrease 34 %) was observed. For carbendazim and thiabendazole, an increase in signal was observed, reaching 45 % and 16 % for commercial nebulizer and 15 % and 29 % for nebulizer design 2. Therefore, on the average, nebulizer Design 2 gives more stable signal in the case of complicated matrices. In our interpretation, this means that the novel nebulizer generates fine spray of droplets that do not contaminate the ion entrance capillary (see the scheme in ref. 14) that much and the MS response is therefore stable in time.

Conclusions

Possible future developments include optimization of the dimensions of the capillaries as well as for example multiple internal assisting capillaries. We also find that this type of nebulizer would be beneficial not only for ESI but also for APCI and APPI, as well as for related technologies such and EESI and DESI.

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