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DRILL: An Electrospray Ionization-Mass Spectrometry Interface for Improved Sensitivity via Inertial Droplet Sorting and Electrohydrodynamic Focusing in a Swirling Flow

Peter A. Kottke[†], Jung Y. Lee[†], Alex P. Jonke[‡], Chinthaka A. Seneviratne[§], Elizabeth S. Hecht[§], David C. Muddiman[§], Matthew P. Torres[‡], and Andrei G. Fedorov^{*,‡,||}

[†]George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, Georgia 30332, United States

[‡]School of Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia 30332, United States

[§]Department of Chemistry, North Carolina State University, Raleigh, North Carolina 27695, United States

^{||}Parker H. Petit Institute of Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, Georgia 30332, United States

Abstract

We describe the DRILL (dry ion localization and locomotion) device, which is an interface for electrospray ionization (ESI)-mass spectrometry (MS) that exploits a swirling flow to enable the use of inertial separation to prescribe different fates for electrosprayed droplets based on their size. This source adds a new approach to charged droplet trajectory manipulation which, when combined with hydrodynamic drag forces and electric field forces, provides a rich range of possible DRILL operational modes. Here, we experimentally demonstrate sensitivity improvement obtained via vortex-induced inertial sorting of electrosprayed droplets/ions: one possible mode of DRILL operation. In this mode, DRILL removes larger droplets while accelerating the remainder of the ESI plume, producing a high velocity stream of gas-enriched spray with small, highly charged droplets and ions and directing it toward the MS inlet. The improved signal-to-noise ratio (10-fold enhancement) in the detection of angiotensin I is demonstrated using the DRILL interface coupled to ESI-MS along with an improved limit of detection (10-fold enhancement, 100 picomole) in the detection of angiotensin II. The utility of DRILL has also been demonstrated by liquid chromatography (LC)-MS: a stable isotope labeled peptide cocktail was spiked into a complex native tissue extract and quantified by unscheduled multiple reaction monitoring on a

*Corresponding Author: agf@gatech.edu; Tel.: +1-404-385-1356; Fax: +1-404-894-8496.

ORCID

David C. Muddiman: 0000-0003-2216-499X

Andrei G. Fedorov: 0000-0003-0859-2541

Notes

The authors declare no competing financial interest.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.anal-chem.7b01555.

Description of electrostatic simulations and DRILL design equations (PDF)

TSQ Vantage. DRILL demonstrated improved signal strength (up to a 700-fold) for 8 out of 9 peptides and had no effects on the peak shape of the transitions.

Graphical Abstract

Following the introduction of the soft ionization technique of electrospray ionization (ESI),^{1–3} mass spectrometry (MS) has assumed a prominent role in biomedical research with applications ranging from fundamental biochemical studies such as protein characterization and kinetic measurements to basic research in cell biology and extending to applied fields such as biomarker discovery, disease diagnosis, and lipid and metabolite profiling.^{4,5} Improvements to electrospray ion sources, sample preparation methods, ion transmission strategies, and mass analyzers have been introduced to further the sensitivity and applications of ESI-MS, yet significant losses of the sampled ions persist.⁶ Therefore, there is room to develop technology to enhance ion production, collection, and transmission and improve the quality of measurements by noise reduction.

Ion production may be primarily improved through alterations in the ion source, for example changing from conventional ESI to nanoESI (nESI),⁷ and through techniques to achieve more complete desolvation. The mechanism of electrospray ionization has been studied extensively for nearly a century, beginning with the parametric spray mode studies of Zelney,⁸ furthered by Taylor's analyses and experiments,^{9,10} applied to mass spectrometry by Dole,³ and made useful for biomolecular MS analysis in the Nobel Prize earning work of Fenn.^{1,2,11} It is an active area of research with ongoing theoretical and experimental work into areas including spray stability, transient spray,^{12,13} and droplet dynamic behavior.¹⁴ In electrospray ionization, a potential is applied to a solution inside of a capillary, producing a strong electric field at the spray capillary tip. This deforms the liquid meniscus into a Taylor cone, at the apex of which electrostatic repulsion forces overcome the surface tension, leading to the expulsion of charged, analyte-containing droplets. The size of the droplet, when pinched off from the Taylor cone, depends on liquid properties,^{14,15} and in the case of nESI (i.e., ESI from tips $\sim 10 \mu\text{m}$ diameter), also depends on the capillary tip diameter.¹⁶ As the parent droplets move through the atmosphere, the electrically neutral solvent in the

droplet evaporates, increasing the charge-to-surface area ratio. When the maximum charge capacity is reached (Rayleigh limit), the droplet undergoes fission.¹⁷ In the fission process, small progeny droplets are ejected from the larger parent droplet, and these progeny droplets, which take only about ~2% of the mass but 15% of the charge, produce most of the fully desolvated “dry” ions and thereby contribute to a higher proportion of detectable ions.¹⁸

In typical ESI, space charge produces an electric field that expands the droplet plume radius with smaller, more highly charged progeny droplets being driven to the outer edge of the plume more rapidly than the larger, less highly charged parent droplets. The expansion of the droplet plume is a source of sample loss because the plume cross-sectional area rapidly becomes much larger than the mass spectrometer inlet.⁶ While sampled amount and MS signal may be increased by decreasing the ESI-to-inlet distance, these gains are limited by the decreased time allowed for desolvation, which results in much of the transmitted current being in the form of clusters or droplets rather than gas phase ions. The importance of desolvation effectiveness in ESI is remarkable, as an estimated 80% of analyte ions that are electrosprayed into the MS inlet are lost to analysis due to incomplete desolvation.⁶ Thus, it is clear that the management of the plume expansion and careful sampling of the most desired fraction (e.g., smaller, highly charged droplets) of the plume are important in ensuring the best sensitivity in ESI-MS.

Efforts have been made to enhance desolvation and sampling by shaping the inlet geometry to increase drag and flow laminarization^{19–21} or via multichannel MS intake capillaries that sample a wider cross section of the electrospray plume.²² Yet, because of the additional functions the MS inlet must fulfill, especially with regards to maintaining vacuum and limiting gas flow, there are constraints imposed on modifying it to improve desolvation. Thus, efforts to enhance desolvation may be more successful in an interface between the ESI source and the MS inlet. The subambient pressure ionization with nanoelectrospray (SPIN)-MS interface relocates emitters to a low pressure environment, thereby decreasing the transmission requirements through the first skimmer and increasing efficiency.²³ Similar attempts have been made to limit the dispersion of ions prior to entering the MS. Interfaces or strategies for better coupling of ESI to the MS inlet have been demonstrated with tactics that can apply different combinations of efforts to (i) increase desolvation rate via increased temperature of the gas surrounding the droplets,^{24–26} (ii) inwardly direct flow to oppose droplet cloud expansion (caused by electrostatic repulsion),²⁷ (iii) have axial flow to reduce time for droplet plume expansion,²⁷ (iv) have selective positioning, e.g., off-axis, of the MS inlet to increase the likelihood of collecting highly charged and mostly desolvated droplets,^{28,29} (v) have electrostatic focusing to combat space charge induced spread,³⁰ and (vi) use a gas curtain (cross-flow) to sweep away neutrals.¹¹ In spite of the significant efforts dedicated to improving ESI to MS inlet transmission and droplet desolvation, studies indicate that there is still room for at least an order of magnitude improvement.³¹ The inability to fully realize this potential sensitivity gain by previous approaches suggests a significantly different strategy may be required.

An ideal interface accomplishes several goals: (i) all charge available in droplets produced by the ion source ultimately ends up on completely desolvated analyte ions, (ii) all charged

analyte molecules enter the MS inlet, (iii) the interface minimizes sample dispersion, and (iv) it prevents entry into the MS of neutral solvent molecules, whether in liquid (droplets) or gas phase. The device described in this paper, DRILL (for dry ion localization and locomotion), is an interface between the ESI ion source and MS inlet that improves sensitivity and detection through manipulation of electrosprayed droplet trajectories.³² DRILL exploits a new approach to charged droplet manipulation in an ESI-MS interface by using inertial sorting via a swirling flow, which allows for an added degree of control not previously available. Through control of electric and flow fields within the DRILL, enabled by independent electric biasing of three electrodes, control of two exit flow paths, and an adjustable convergent swirling flow generator, multiple modes of operation are possible, which accomplish one or more above-mentioned goals. Ultimately, the goal of DRILL is to produce from each analyte molecule in solution a desolvated, i.e. “dry”, ion and to selectively translate those ions to the mass spectrometer inlet without losses.

In this work, we investigate DRILL performance in a mode that removes larger droplets while accelerating the remainder of the ESI plume toward the MS inlet. Our analysis of DRILL performance using flow and droplet transport simulations suggests that the primary role played by DRILL is removal of large droplets from the ESI plume. Larger drops are removed because of the intense swirling flow, which allows only smaller droplets to leave the device. As a result, DRILL directs a stream of very small highly charged droplets at the MS inlet with a resulting increased signal-to-noise ratio (SNR) and improved levels of detection. In this mode, which has all of the gas flow directed out the front of the DRILL toward the MS inlet, i.e., “forward-only” flow, we demonstrate that DRILL provides significant benefits in terms of analyte detection in direct infusion (DI)-ESI-MS and liquid chromatography (LC)-ESI-MS experiments on different mass spectrometers obtained in two different laboratories.

EXPERIMENTAL SECTION

DRILL Device Fabrication and Description

The DRILL is a roughly cylindrical device machined primarily from PEEK and aluminum: the total length of the device is 15 cm, and the diameter of the larger back cylinder is 5 cm (Figure 1A). The device has two main sections: the flow-creation back section, which allows for gas and pressure gage connection and creates a swirling flow (Figure 1B) which is then transmitted through an adjustable converging flow annulus into the droplet sorting and desolvation unit, and the droplet sorting and desolvation unit, which has quartz windows for electrospray observation and three electrically conducting parts (electrodes) which allow manipulation of the electric field within the section without altering the electric field at the spray tip from that required for electrospray (Supporting Information).

Gas flow to DRILL is via a port at the back and can be from any desired pressurized gas source. A Swagelok backpressure regulator is used to step down nitrogen from a cylinder or nitrogen generator to under 20 kPa gauge pressure. Pressure in DRILL is monitored via a low pressure gauge (max range 3 psig). The nitrogen flow in DRILL is directed through slots in a swirler (Figure 1A) so that the flow acquires a strong swirling characteristic. The angular velocity of the gas increases (Figure 1B) due to momentum conservation as the flow

space converges while moving from the flow creation section to the droplet sorting and desolvation section and again as the flow enters the electrode tubes. High voltage power supplies (Stanford Research Systems PS350) maintain the electrical potentials of the front and back electrode tubes and the outer shell electrode. The front and back electrode tubes are 0.125" OD/0.055" ID stainless steel tubes, 2 and 15 cm in length, respectively, and are sealed in place with Upchurch removable fittings (F-368) so that the electrode tubes' extension into the droplet sorting and desolvation region can be adjusted. All interfaces are sealed with O-rings or via compression fitting ferrules.

DRILL Parameters

DRILL electrical conditions for a given experiment are specified by four potentials applied to electrodes shown in Figure 1A: the potential applied to the nESI emitter, V_{spray} , the potential applied to the outer shell electrode, V_{shell} , the potential applied to the back electrode tube, V_{back} , and the potential applied to the front electrode tube, V_{front} . The geometric configuration of DRILL, which is highly adjustable, is defined by the position of the nESI emitter in the desolvation unit, the extent the swirler cone protrudes into the desolvation unit, and the positions of the front electrode tube and the back electrode tube. The flow conditions in DRILL depend on the geometric configuration and also on the supplied gas pressure, which is kept between 0.05 and 0.5 psig, and the flow restriction by the back flow control valve, which is closed in the experiments reported in this work ("forward-only" flow mode). DRILL is mounted on an xyz stage so that its position relative to the MS inlet can be controlled.

Simulations

A simplified model of the dominant physics in DRILL was developed so that tractable numerical simulations could be used to understand DRILL behavior. The assumptions used for the model are (1) space charge effects are negligible in their impact on droplet trajectories, (2) fluid flow remains laminar, (3) droplet–droplet interactions are negligible, as is the effect of droplets on the surrounding fluid flow patterns, and (4) DRILL response is quasi-steady. Justifications for these assumptions are provided in the Supporting Information. With these assumptions, a DRILL model was developed and solved using ANSYS-FLUENT, a flexible computational fluid dynamics program previously demonstrated for ion source and interface modeling.^{33–35} The fluid flow model uses pressure inlet and outlet boundary conditions for the steady state, laminar, incompressible Navier–Stokes equations to obtain a the gas flow field. The electric field within DRILL is found via solution of the Laplace equation (using FLUENT's user defined scalar governing equation with no advection) with specified electric potentials on electrode surfaces and zero potential gradient normal to dielectric surface boundaries. Droplet trajectories are obtained using the discrete phase model with an added user defined body force to include electric forces on the droplets.

Reagents

Angiotensin I human acetate salt hydrate, angiotensin II, and formic acid were purchased from Sigma-Aldrich (St. Louis, MO). Coated fused silica PicoTip emitters (360 μm OD, 75 μm ID, 30 μm tip ID) were purchased from New Objective (Waltham, MA). Unless

otherwise specified, all solvents used were of HPLC grade and purchased from Honeywell-Burdick & Jackson (Muskegon, MI). Low-purity nitrogen was used as the gas-flow source.

Direct Infusion Experiments

For the direct infusion SNR determination with and without DRILL on the TSQ Vantage (Thermo Fisher Scientific, Waltham, MA) triple quadrupole mass spectrometer, angiotensin I was infused at 400 nL/min in 50:50 water:methanol with 0.1% formic acid into the DRILL ($V_{\text{spray}} = 2750$ V, $V_{\text{shell}} = 900$ V, $V_{\text{back}} = 2100$ V, $V_{\text{front}} = 500$ V, ESI emitter extension 9.7 mm, swirler cone extension 3 mm, front electrode tube extension 7.5 mm, back electrode tube extension 3 mm, nitrogen supply pressure 0.5 psig, back flow control valve closed, distance to MS inlet 4.5 mm). The QqQ was operated in full scan mode to assess the noise in the sample spectrum with a range of 300–1000 m/z and inlet capillary temperature of 275 °C.

Direct infusion limit of detection experiments were carried out with a standard breadboard, a Thermo Fisher Scientific nanospray flex ion source (FLEX), and the DRILL on an LTQ Orbitrap (Thermo Fisher Scientific, Waltham, MA) using solutions of angiotensin II from 0.1 to 50 nM in LC-MS grade solvents (Fisher Scientific, Waltham, MA). For direct infusion, the emitter was coaxial with the MS inlet and position and applied voltage were optimized to obtain stable spray and consistently high signal. For each concentration/method, runs were performed in triplicate (with the exception of the three highest concentrations of direct infusion, which had only duplicate runs). Conditions used included: $V_{\text{spray}} = 2500$ V, $V_{\text{shell}} = 600$ V, $V_{\text{back}} = 2000$ V, $V_{\text{front}} = 500$ V, nESI emitter extension 8.5 mm, swirler cone extension 3.5 mm, front electrode tube extension 7.5 mm, back electrode tube extension 0 mm, nitrogen supply pressure 0.2 psig, back flow control valve closed, and the DRILL was aligned coaxial with respect to and 5 mm away from the inlet capillary of the mass spectrometer. The FTMS scan mode was selected with a range of 150–2000 m/z and inlet capillary temperature of 200 °C. AGC was set to 5E5 with an injection time limit of 100 ms. To compare ion abundances, we normalized results for injection time.

LC-MS Experiments

Stable isotope-labeled (SIL) $C^{13}N^{15}$ peptides were synthesized by the Mayo Clinic Proteomics Research Center (Rochester, MN) and spiked into a wild-type crude xylem protein extraction prepared as previously described.³⁶ Sample (10 fmol/peptide) was injected onto a 23 cm C18 (2.6 μm particles with 100 Å pore size) column (100 μm ID capillary, in-house made frit). LC-MS was performed using an Eksigent nanoLC-2D system coupled to a TSQ Vantage using a 0–40% 98:2:0.1 acetonitrile:water:formic acid gradient. Unique conditions to the DRILL-TSQ interface included: $V_{\text{spray}} = 4000$ V, $V_{\text{shell}} = 900$ V, $V_{\text{back}} = 1900$ V, $V_{\text{front}} = 400$ V, nESI emitter extension 6 mm, swirler cone extension 0.6 mm, inlet temperature = 350 °C. For multiple reaction monitoring (MRM), unique transitions were monitored unscheduled as previously described,³⁶ leading to the identification of nine SIL peptides.

RESULTS AND DISCUSSION

DRILL Theory

The fundamental concept behind DRILL is based on momentum conservation, which requires that droplet trajectories are determined by a combination of electric field force, drag force, and inertia. Analysis of DRILL is similar to analysis of cyclone separators except that particle sizes are smaller than those for a typical separator, the flow in DRILL is expected to be primarily laminar (see Supporting Information), and DRILL has the added complexity of electric fields and forces not usually employed in cyclone separators.³⁷ In swirling flow, inertia results in an apparent outwardly directed pseudoforce, a centrifugal force. Each of the three forces (including the pseudocentrifugal force) has a different dependence on droplet size and also depends on different “driving fields” (electric field, total velocity, and angular or “swirl” velocity). As a result, it is possible to set up combinations of electric fields and flows in DRILL that prescribe different fates for droplets based on their size. Centrifugal forces have the strongest dependence on droplet size, scaling with the radius to the third power. The electric force is the product of the charge and the electric field, and the charge on a droplet is limited by the Rayleigh charge limit, which scales as droplet radius to the 1.5 power. To leading order, drag is proportional to the droplet radius. Eqs 1–3 express these relationships:

$$F_C = \frac{\frac{4}{3}\rho R^3 v_\theta^2}{r} \quad (1)$$

$$\vec{F}_E = 8\pi \sqrt{\epsilon_r \epsilon_0} R^{1.5} \vec{E} \quad (2)$$

$$\vec{F}_D = 6\pi\mu R \vec{v}_{\text{rel}} \quad (3)$$

where F_C is the strength of the centrifugal pseudoforce, ρ is the droplet density, R is the droplet radius, v_θ is the angular or “swirl” velocity, r is radial position of the droplet from the axis of revolution for the swirling flow, $\vec{F}_E$ is the electric force for a droplet at the Rayleigh charge limit, ϵ_0 is the relative electrical permittivity of the gas around the droplet, ϵ_0 is the electrical permittivity of free space, $\vec{E}$ is the electrical field at the droplet location, $\vec{F}_D$ is the drag force on the droplet assuming Stoke’s drag law is accurate, μ is the kinematic viscosity of the gas around the drop, and $\vec{v}_{\text{rel}}$ is the relative velocity between the droplet and the carrier gas. It is particularly important to note, when considering DRILL performance, the inverse relationship with radial position, r , in eq 1. Especially because conservation of angular momentum tends to result in increasing angular velocity v_θ with decreasing r , the centrifugal effect becomes very strong along the DRILL centerline and decays rapidly away from the centerline. Thus, the inertial sorting effect in DRILL is primarily seen through exclusion of larger droplets from positions close to the DRILL axis,

so that only smaller, highly charged droplets are preferentially extracted from DRILL through the front electrode tube to MS inlet. DRILL parameters for targeted droplet filtering can be determined from theoretical considerations: the equations that govern DRILL design and operation along with experimental validation of DRILL theory are provided as Supporting Information.

An unusual and important feature of DRILL is the presence of multiple electrodes. These allow the electric field in the DRILL to be changed while maintaining the conditions required for electrospray, i.e., by changing the potential differences between the spray tip, back electrode tube, outer shell electrode, and front electrode tube, the direction and magnitude of electric field throughout DRILL can be altered without changing local character of the electrospray.

DRILL Simulations

The fate of electrosprayed droplets in DRILL is the result of a complex interplay of electric field, droplet dynamics, heat and mass transfer, and gas flow. The output of greatest interest, evolution trajectories for a given droplet size, is obtained using FLUENT's discrete phase model (Figure 2), with a user defined body force employed to implement the electric force (eq 2). For conditions used in the described experiments, simulations show that inertial forces prevent droplets larger than about 3 μm in diameter from leaving DRILL. Instead, these larger droplets impinge on the front electrode tube, e.g., Figure 2D. For smaller droplets, on the other hand, electric field (near the emitter tip) and then drag forces dominate throughout DRILL, e.g., Figure 2C.

Computational fluid dynamics/multiphysics simulations provide the ability to interrogate and understand the complex interplays within the device and thereby to elucidate the mechanisms for noise reduction that lead to enhanced detection with DRILL. Simulations of DRILL were used to compare expected charged droplet fate as a function of droplet size (Figure 2) and demonstrate that for conditions used in experiments, DRILL acts as a droplet size filter. DRILL allows small diameter droplets to exit while removing larger droplets from the ESI plume. The fundamental physical basis for this filtering effect is the difference in inertia of the droplets. The trajectory of electrosprayed droplets is usually due to the interplay of electric and drag forces, with inertia playing a negligible role due the extremely small droplet size. However, due to the strong swirling flow near the DRILL centerline (one can envision a fast-spinning tornado-like flow at the entrance to the front electrode tube), inertia can become dominant in the form of the centrifugal force, flinging large droplets away from the tube entrance. At the same time, gas flows from the DRILL front electrode tube at a high velocity, up to 70 m/s, which reduces the time for spreading of the droplet plume between the DRILL exit and MS inlet. The impact of DRILL is therefore to "invert" the ESI plume (i.e., bring smaller, highly charged droplets toward the centerline while pushing the large, low charge droplets to the periphery) from that normally observed in ESI and to extract the information-rich, highly charged, small progeny droplets for MS analysis. Thus, DRILL sensitivity enhancement observed in experiments is due to the synergistically combined effects of inertial sorting via rotational flow and hydrodynamic focusing.

Direct Infusion Experiments

The initial assessment of DRILL impact was accomplished via quantification on two different mass spectrometers that operate using different mass analyzers (quadrupole versus orbitrap). On the TSQ Vantage, an experiment with angiotensin I was performed to assess the SNRs of a standard DI compared to the DRILL. Blank ESI solutions (water:methanol, 50:50, 0.1% formic acid) were infused in positive ion mode, and Figure 3A shows the corresponding mass spectra for both direct infusion ESI and DRILL-ESI. Several ambient background ions were detected at higher abundance levels with direct infusion compared to detection with DRILL, and spectra from DRILL showed considerably fewer background peaks than direct infusion, suggesting that analysis with DRILL will enable detection of signals from less abundant analytes. This translated to reduced chemical noise and higher SNRs using the DRILL. The SNR was calculated based on the peak-to-peak noise amplitude (N_{pp}) using the signal height (S_H) at the center of the +3 charge state of angiotensin I ($m/z = 432.89$).³⁸ A 10-fold increase was observed in the SNR in DRILL (Figure 3C) compared to direct injection (Figure 3B).

The limit of detection of angiotensin II was subsequently assessed on the LTQ Orbitrap. Figure 4A compares the peak intensity for DRILL, DI, and the nanoFlex ion source as a function of angiotensin II concentration from 0.1 to 50 nM. Though the total ion current from the DRILL was significantly lower, the LOD for this model peptide was improved by an order of magnitude with the DRILL. Comparisons were made on the basis of total ion counts, accounting for the fact that the injection time to meet the target AGC was slower when using the DRILL compared to that using the DI or nanoFlex sources. As in the TSQ experiments, the increase in signal is attributed to noise reduction. Figure 4B shows subset of the mass window encapsulating the +2 angiotensin II peak; the DRILL spectrum exhibits considerably less local noise. The ability of DRILL to improve the LOD by reducing noise when quantifying with high-resolving power instrumentation demonstrates the challenge of ambient and chemical noise present even in simple mixtures. Figure 4C shows the ratio of the target analyte peak intensity to the sum of the top 20 nonanalyte peak intensities for DRILL, DI, and the nanoFlex and shows that DRILL exhibits relatively less interference from ambient or solvent effects.

LC-MS/MS Experiments

Multiple reaction monitoring is a typical approach used with triple quad mass spectrometers for sensitive and selective quantification of targeted analytes. However, while attomole detection limits may be achieved with careful optimization, those methods often include fewer transitions, increasing the error associated with quantification. Having characterized DRILL benefit for peptide detection enhancement, we used MRM-LC-ESI-MS with and without DRILL to test the applicability of the DRILL to more typical analyses. The overall chromatogram for the standard LC-infusion had significantly more background across all regions (Figure 5A). It was interesting that for an LC application with a complex proteome background, the DRILL still appeared to filter for undesirable noise. For the 9 SIL peptides, significant gains were observed for 8 of the species when analyzed with the DRILL (Figure 5B) with up to a 700-fold increase calculated. Interestingly, the trends in abundances did not correlate with the charge states of the precursors (+2 or +3), their neutral mass, retention

time, or their peptide sequences. More investigation to understand these differences is underway. The resolution of the peaks was identical between DRILL and nonDRILL methods.

Use of DRILL with all flow directed out the front electrode tube, i.e., with no reverse flow, results in enhanced SNR and therefore improved LOD for peptides angiotensin I and II, Figures 3 and 4 and for SIL peptides spiked into a complex proteome Figure 5. The improved SNR comes despite lower overall ion currents. Instead, in MS¹ experiments, DRILL reduces noise to such a degree that analyte ions can be detected at concentrations lower than those without DRILL. In other words, DRILL in this mode is not increasing the overall collection efficiency of electrosprayed ions. Furthermore, on the basis of the comparison of injection times on the LTQ Orbitrap with and without DRILL, DRILL in this operational mode does not increase the transmission and collection efficiency of the target analyte ions. Increasing the ion transmission and collection efficiencies without sacrificing the SNR gain enabled by DRILL is a promising next step for DRILL optimization.

The peptides used for initial sensitivity analysis, where DRILL showed a significant benefit, are known to “fly well”, i.e., they are amenable to ESI-MS analysis. Such analytes typically have relatively good interfacial activity, preferentially partitioning to the droplet surface and then into progeny droplets. For similar reasons, DRILL was also very successful in enhancing sensitivity in LC-MS experiments. However, there are many ESI-MS experimental workflows in which target analytes are difficult to detect because of the effects of the analyte physicochemical interactions with the solvent. Some specific examples include native mass spectrometry of full length proteins (i.e., top down proteomics), lipidomics, and analysis of glycans. At least some of the difficulty in these analyses is attributed to solvophilicity of the analytes such that they are systemically retained in parent droplets during droplet fission. The key to improved sensitivity in such applications will be prescribing a different fate for retained parent droplets: the loss of these droplets is the main transmission loss in DRILL in “forward-only” mode. The fate of droplets within DRILL is the result of a complex interplay of forces and phenomena, and experimental validation of the simulation results is expected to require careful engineering characterization. With a validated DRILL design model, we expect to be able to create combined recirculating flows and droplet redirecting electric fields so that parent droplet retention time in DRILL can be significantly prolonged, thus allowing for a number of successive fission events and extending the range of potential target analytes. Additional improvement in DRILL application is expected to be obtained through design of experimental methods, which have been shown to be extremely successful in optimizing ESI-MS methods.³⁹

CONCLUSIONS

DRILL was introduced as a successful interface for ESI-MS, improving SNR and limit of detection in direct infusion of angiotensin I and II and sensitivity of LC-MS-MRM analysis of stable isotope labeled peptides in a protein extract. Numerical simulation of DRILL suggests that improved performance occurs via selection of smaller droplets from the ESI plume via inertial separation, followed by hydrodynamic drag guided transport to the mass spectrometer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Fenn JB. *Angew Chem, Int Ed.* 2003; 42:3871–3894.
2. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. *Science.* 1989; 246:64–71. [PubMed: 2675315]
3. Dole M, Mack L, Hines R, Mobley R, Ferguson L, Alice MD. *J Chem Phys.* 1968; 49:2240–2249.
4. Mano N, Goto J. *Anal Sci.* 2003; 19:3–14. [PubMed: 12558016]
5. Finehout EJ, Lee KH. *Biochem Mol Biol Educ.* 2004; 32:93–100. [PubMed: 21706701]
6. Page JS, Kelly RT, Tang K, Smith RD. *J Am Soc Mass Spectrom.* 2007; 18:1582. [PubMed: 17627841]
7. Wilm M, Mann M. *Anal Chem.* 1996; 68:1–8. [PubMed: 8779426]
8. Zeleny J. *Phys Rev.* 1917; 10:1–6.
9. Taylor G. *Proc R Soc London, Ser A.* 1966; 291:145–158.
10. Taylor G. *Proc R Soc London, Ser A.* 1969; 313:453–475.
11. Yamashita M, Fenn JB. *J Phys Chem.* 1984; 88:4451–4459.
12. Stark JPW, Alexander MS, Smith KL. *J Appl Phys.* 2014; 115:044905.
13. Marginean I, Nemes P, Vertes A. *Phys Rev Lett.* 2006; 97:064502. [PubMed: 17026172]
14. Collins RT, Sambath K, Harris MT, Basaran OA. *Proc Natl Acad Sci U S A.* 2013; 110:4905–4910. [PubMed: 23487744]
15. Gañán-Calvo A, López-Herrera J, Rebollo-Muñoz N, Montanero J. *Sci Rep.* 2016; 6:1. [PubMed: 28442746]
16. Juraschek R, Dülcks T, Karas M. *J Am Soc Mass Spectrom.* 1999; 10:300–308. [PubMed: 10197351]
17. Rayleigh L. *Philos Mag.* 1882; 14:184–186.
18. Kebarle P, Tang L. *Anal Chem.* 1993; 65:A972–A986.
19. Pauly M, Sroka M, Reiss J, Rinke G, Albarghash A, Vogelgesang R, Hahne H, Kuster B, Sesterhenn J, Kern K, Rauschenbach S. *Analyst.* 2014; 139:1856–1867. [PubMed: 24479126]
20. Krutchinsky AN, Padovan JC, Cohen H, Chait BT. *J Am Soc Mass Spectrom.* 2015; 26:649–658. [PubMed: 25588722]
21. Krutchinsky AN, Padovan JC, Cohen H, Chait BT. *J Am Soc Mass Spectrom.* 2015; 26:659–667. [PubMed: 25667060]
22. Kim T, Tang K, Udseth HR, Smith RD. *Anal Chem.* 2001; 73:4162–4170. [PubMed: 11569805]
23. Page JS, Tang K, Kelly RT, Smith RD. *Anal Chem.* 2008; 80:1800–1805. [PubMed: 18237189]
24. Chowdhury SK, Katta V, Chait BT. *Rapid Commun Mass Spectrom.* 1990; 4:81–87. [PubMed: 2134340]
25. Covey, TR., Anacleto, JF. US Patent. 5,412,208. 1995. Ion spray with intersecting flow
26. Henion, JD., Covey, JR., Bruins, AP. US Patent. 4,861,988. 1989. Ion spray apparatus and method
27. Hawkrige AM, Zhou L, Lee ML, Muddiman DC. *Anal Chem.* 2004; 76:4118–4122. [PubMed: 15253651]
28. Smith RD, Shen Y, Tang K. *Acc Chem Res.* 2004; 37:269–278. [PubMed: 15096064]
29. Bruins AP, Covey TR, Henion JD. *Anal Chem.* 1987; 59:2642–2646.

30. Schneider BB, Douglas DJ, Chen DDY. J Am Soc Mass Spectrom. 2002; 13:906–913. [PubMed: 12216730]
31. Cech NB, Enke CG. Mass Spectrom Rev. 2001; 20:362–387. [PubMed: 11997944]
32. Fedorov, AG. US Patent. 7,595,487. 2009. Confining/focusing vortex flow transmission structure, mass spectrometry systems, and methods of transmitting particles, droplets, and ions
33. Forbes TP, Degertekin FL, Fedorov AG. J Am Soc Mass Spectrom. 2010; 21:501–510. [PubMed: 20149681]
34. Forbes TP, Degertekin FL, Fedorov AG. Phys Fluids. 2011; 23:012104.
35. Garimella S, Xu W, Huang G, Harper JD, Cooks RG, Ouyang Z. J Mass Spectrom. 2012; 47:201–207. [PubMed: 22359330]
36. Shuford CM, Li Q, Sun YH, Chen HC, Wang J, Shi R, Sederoff RR, Chiang VL, Muddiman DC. J Proteome Res. 2012; 11:3390–3404. [PubMed: 22524869]
37. Dietz PW. Powder Technol. 1982; 31:221–226.
38. Müller M, Schiller J, Petkovi M, Oehrl W, Heinze R, Wetzker R, Arnold K, Arnhold J. Chem Phys Lipids. 2001; 110:151–164. [PubMed: 11369324]
39. Hecht ES, Oberg AL, Muddiman DC. J Am Soc Mass Spectrom. 2016; 27:767–785. [PubMed: 26951559]

Figure 1.

(A) DRILL is an interface between an ESI or nESI emitter and the mass spectrometer inlet, constructed primarily of PEEK (beige) and aluminum (light gray). Charged droplets and dry ions exit the DRILL interface front electrode tube, which is positioned in front of and in line with the MS inlet. DRILL is constructed in an adaptable manner with multiple adjustments possible and a modular design, allowing easy exchange of components as well as disassembly for cleaning. The ESI emitter, which is affixed to the DRILL top via an exchangeable adapter, electrosprays the sample into the DRILL due to an electric field induced by the combined effects of potentials applied to three individually controlled electrodes: the outer shell electrode, the front electrode tube, and the back electrode tube. The width of the converging adjustable annulus between the inner cone and the outer converging joint is changed via a threaded union which permits fine-tune positioning (green arrow) of the inner cone. Gas flow partitioning between the front and rear electrode tubes is controlled by a valve connected to the back of the rear electrode tube (not shown). (B) Gas flow region in DRILL: red dotted outline corresponds to that in A. Flow simulation results visualized via path lines (colored curves) and velocity vectors show the increasing velocity of the swirling flow due to converging geometry from the swirler to the front electrode tube. The region outlined in blue dashes is the region depicted in Figure 2.

Figure 2.

Simulated droplet fates demonstrating the roles of different forces. (A) Droplets with zero charge and mass follow the fluid flow, indicated via the vector arrows on the symmetry plane of DRILL, and would all be removed from DRILL. (B) Charged, massless droplets with zero drag coefficient would be driven to the outer shell by the electric field within DRILL; in this contour plot of electric potentials on the DRILL symmetry plane, the electric field moves droplets in the direction of the steepest descent. With all forces considered, droplet fate depends on size. In panels C and D, the solid colors indicate the domains of dominance by the inertial effect that leads to the centrifugal pseudoforce given by eq 1 (yellow), the electric force given by eq 2 (blue), or the drag force given by eq 3 (green).

Figure 3.

(A) Comparison of the mass spectra of a blank solution (methanol:water (50:50) with 0.1% formic acid) with and without DRILL showed large reductions in the background. Sample mass spectra of 25 nM angiotensin I by direct infusion (B) without and (C) with the DRILL. The SNR was calculated according to the peak-to-peak formula using the signal height of the $+3$ charge state of angiotensin (S_H) against the background in two different m/z ranges each spanning 100 Th (insets).

Figure 4.

Improvement in limit of detection and signal-to-noise observed with DRILL compared to standard nano-ESI. (A) LOD study of angiotensin II peptide concentrations ranging from 0.1 to 50 nM using DRILL, no DRILL (DI), and nanospray Flex (FLEX) ion sources. Data represent measurement of the $[M + 2H]^{2+}$ charge state in all cases. (Inset) Magnified view of concentration range 0.1–2 nM, highlighting distinct sensitivity advantage observed with DRILL. (B) Local noise level comparison for angiotensin II observed with and without DRILL. (left) Wide spectrum view showing target and nontarget ion signals. (right) Magnified view of angiotensin isotope cluster and surrounding noise observed with and without DRILL. (C) A comparison of ratios for the angiotensin II peak abundance relative to the sum of the top 20 nontarget peak intensities observed for each of the three ionization

sources, showing that DRILL improves trap filling productivity (target over non target ions) by ~2-fold compared to the other tested ion sources without DRILL.

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Figure 5.

Evaluation of DRILL performance for LC-MS/MS in MRM mode. (A) Base peak chromatograms of the SIL peptide mixture. (B) The unique MRM transitions³⁶ for the nine SIL peptides were quantified in Skyline. The summation of transitions detected in both methods (DI and DRILL) is reported.