A Combination MAI and MALDI Vacuum Source Operational from Atmospheric Pressure for Fast, Robust, and Sensitive Analyses

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ABSTRACT: Previously, vacuum matrix-assisted ionization (vMAI) was employed with matrix:analyte sample introduction into the vacuum of the mass spectrometer on a probe sample introduction device. Low attomole detection was achieved while no carryover was observed even for concentrated samples. Here, we report a new vacuum ionization source designed to duplicate the sensitivity and robustness of probe device while providing fast multi-sample introduction to vacuum and rapid sequential ionization. Exposure of a sample to the vacuum of the mass spectrometer provides spontaneous ionization of volatile as well as nonvolatile analytes without the need of external energy input. However, the novel source design described herein, in addition to vMAI, can employ a laser to obtain vacuum matrix-assisted laser desorption/ionization (vMALDI). In particular, ionization by vMAI or vMALDI is achieved by using the appropriate matrix. Switching between ionization modes is accomplished in a few seconds. We present results demonstrating the utility of the two ionization methods in combination to improve molecular analyses of sample composition. In both ionization modes, multiple samples can be sequentially and rapidly acquired to increase throughput in MS. With the prototype source, samples were acquired in as little as 1 second per sample. Exchanging multi-sample plates can be accomplished in as little as 2 seconds suggesting low-cost high throughput automation when properly developed.

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Introduction

Currently, the traditional ionization technologies of electrospray ionization (ESI), matrix-assisted laser desorption/ionization (MALDI), and various ambient ionization methods are commercially available and widely used in mass spectrometry (MS) laboratories.[1–4] Vacuum time-of-flight (TOF) mass spectrometers provide excellent ion transmission in MALDI, however, can interfere with the analysis of small molecules. The utility of MALDI for high mass analyses on commonly available atmospheric pressure ionization (API) mass spectrometers is limited because of mass range issues related to the formation of singly charged ions.[5] While ESI produces multiply charged ions allowing the analysis of high-mass compounds with API mass spectrometers, it has limitations with direct analysis of biological fluids and other 'dirty' or salty samples, primarily because of suppression of ionization, and ion source, or instrument contamination. Atmospheric pressure (AP)-MALDI has been demonstrated on API mass spectrometers,[6–8] but is less sensitive than vacuum MALDI.[9] Nevertheless, AP-MALDI has the advantage of operation on instruments also having ESI capability.[10,11] However, these ionization technologies, including the newer developments of ambient ionization,[12–15] have retained some limitations such as need for high-voltage, specialized hardware, operator expertise, and, for some, a laser.

Attractive alternatives are *inlet* and *vacuum* ionization methods.[16–21] Vacuum matrix-assisted ionization (vMAI) does not require a laser,[22–24] but, like laserspray ionization (LSI)[25–30] and MALDI,[1,2,31] uses a matrix. In vMAI, a specific matrix, or a matrix mixture,[17,32] is used which spontaneously converts compounds of the analyte associated with the matrix into gas-phase ions. This ionization process occurs during the sublimation of the matrix,[22,33] and produces multiply charged ions, like ESI. In some cases, vMAI has produced higher sensitivity and selectivity compared to ESI or vacuum MALDI-TOF.[24,32] The ability to acquire 24 samples by vMAI in 4 minutes, disregarding the *ca.* 2 minutes to introduce the sample plate into the vacuum of the mass spectrometer, was demonstrated on a Waters SYNAPT G2S mass spectrometer.[34] Matrix combinations (mixtures)[16,35–39] or additives,[40] such as ammonium salts,[41–43] can be utilized to enhance the effectiveness of analyte ionization providing more specificity or breadth and depth analyzing complex samples. The binary matrices can be combined in various molar ratios as shown in previous studies to enhance a desired attribute.[16,24,32,44] Fundamental studies of the new ionization processes have been reported.[16,17,37,45]

With vMAI, proteins as large as bovine serum albumin (BSA) (66 kDa) were observed without engaging the laser using commercial intermediate pressure MALDI and ESI and atmospheric pressure chemical ionization (APCI) sources on API mass spectrometers made possible because of the formation of multiply charged ions.[22,45] Examples of biomedical and clinical relevance include, as representative examples, analysis of drugs of abuse,[46] metabolites,[47,48] gangliosides, membrane proteins, and various toxins and other potential health hazard materials.[49] Quantification of proteins[50] and drugs,[46] as well as bacterial differentiation have been demonstrated.[34,45,51] Because of multiply charging, high performance mass spectrometers with well-known advantages such as mass accuracy, mass resolution, as well as other advanced measurement technologies such as electron transfer dissociation (ETD)[29,52–54] are applicable. As samples become increasingly complex, high performance mass spectrometers become more critical, as does advanced data analyses for accurate and fast analyses.[5,55,56]

The construction of a single-probe vMAI source for a Thermo Q-Exactive Focus mass spectrometer, and its use in the analyses of biological fluids, bacterial extracts, tissues, and high concentration samples showed no indication of inlet or instrument contamination.[55] Multiply charged analyte ions were spontaneously produced during matrix sublimation.[22,24,32,55] Despite excellent sensitivity and robustness to carryover and instrument contamination, the performance is limited because the single-probe vMAI ion source is not suitable for automation or high throughput.[55,57] Here, we describe for the first time a fundamentally new ion source and its ability to rapidly sequentially expose matrix:analyte samples individually to the vacuum of the mass spectrometer such that ions and charged particles produced from the sample, using vMAI or vMALDI, traverse from the sample surface into the mass analyzer of the mass spectrometer for detection and analysis. To our knowledge, this is also the first multi-mode ionization apparatus based on *vacuum* ionization. Sensitivity, robustness, speed, and simplicity have been demonstrated. Further, the multi-mode source is easily exchanged with the commercial ESI or APCI sources. The advantages of having multi-mode ionization methods rapidly available on the same instrument, especially with respect to extending the breadth of detection of different classes of compounds is discussed.

Experimental

Materials

HPLC grade water, acetonitrile (ACN), methanol (MeOH), acetone, synthetic urine, formic acid, hydroxychloroquine sulfate (HCQ), >98% purity, polyethylene glycol 2000 (PEG 2000), angiotensin I, angiotensin II, erythromycin, gramicidin S, bradykinin, 3-nitrobenzonitrile (3-NBN), 2′,5′-dihydroxyacetophenone (2,5-DHAP), α-cyano-4-hydroxycinnamic acid (CHCA), 2,5-dihydroxybenzoic acid (2,5-DHB), and methyl 3-nitrobenzoate were purchased from Sigma Aldrich (St Louis, MO). BSA trypsin digest was purchased from Waters (Milford, MA) 10 years ago. 1-Propionyl-lysergic acid diethylamide (1P-LSD), 6-allyl-6-nor-LSD (AL-LAD) and lysergic acid 2,4-dimethylazetidide (LSZ) were purchased from Cerilliant (Round Rock, TX). Analysis of fungus from a strawberry grown in-house. An at least 11-year-old nitrogen laser (337 nm, Spectra Physics VSL-337ND-S) from Spectra Physics (Santa Clara, CA) was used. Positively charged microscopes slides were purchased from BioLink Laboratories (Washington, DC). In some cases, stainless steel sample plates were used.

Stock solutions of the analyte were prepared individually in ACN or MeOH (1P-LSD, Al-lad and LSZ). Analyte solutions were further diluted with ACN:water to make concentration ranging from 2 to 5 pmol μL^{-1}, and 10 pmol μL^{-1} for bovine serum insulin. For the detection of 1P-LSD and HCQ in synthetic urine, a standard (1000 ppm) LSD solution was prepared in methanol and HCQ in ACN:water and added to synthetic urine to provide 1 pmol μL^{-1} and 3 pmol μL^{-1} solutions. Matrices were dissolved in 80:20 ACN:water or pure methanol at 50 mg mL^{-1} concentration. For vMAI binary matrices, 2,5-DHAP:3-NBN, methyl 3-nitrobenzoate:3-NBN and CHCA:3-NBN were prepared at 8:2 *v*/*v* ratio. For vMALDI, 2,5-DHAP:CHCA was prepared at 1:1 *v*/*v* ratio.

Mass Spectrometry

A Thermo Fisher Scientific (Bremen, Germany) Q-Exactive Focus mass spectrometer was used. The prototype multi-mode vacuum ionization source was designed to replace the instrument's ion source and inlet optics as depicted with a photograph and cross section view (**Figure 1**). The flange assembly comprises an opening (hole) with a diameter of 0.63 cm, the flange's guide rails (spaced apart ca. 25 mm) which holds a valve plate and/or spacer plate in place, a heated metal tube or heated ion guide (~50 °C for studies described herein) placed between the inlet opening and the S-lens of the instrument. The hole of the flange establishes fluid communication between a higher-pressure region, preferably AP, and a lower sub-AP pressure associated with the normal mass spectrometer operation and its vacuum pumps allowing ions and charged matrix:analyte particles into the mass analyzer.[37,58–60]

The spacer plate is typically a metal block ranging from ~2 - 13 mm thickness that can be moved along the guide rails of the flange. There are multiple holes drilled through the spacer plate, typically with a channel diameter smaller than the flange opening. Matrix:analyte sample spots are deposited on, for example, a microscope glass slide from solution at the interval distance of the channels in the spacer plate so that alignment with the channels is established. Microscope glass slides with multiple matrix:analyte samples can then be attached onto one side of the spacer plate while the other side of the spacer plate slides along the guide rails against a flat surface of the flange. When the hole in the flange and channel in the spacer plate are aligned, matrix:analyte samples are exposed to the vacuum of the mass spectrometer. Only one matrix:analyte sample spot is exposed to the vacuum at a time. Moving towards the next sample spot, the hole opening to the mass spectrometer is "closed off" by the area between two spacer plate channels. The matrix:analyte sample does not come in direct contact with the flange surface which otherwise could result in carryover between samples. Typically, +60 V to +200 V is applied on the spacer plate to improve extraction efficiency of ions and charged clusters. The pressure differential between AP and the mass spectrometer vacuum holds all plates together when the sample plate covers the holes in the spacer plate to achieve a pressure in the first vacuum region of <1 mbar. The source pressure, when a blank sample plate assembly is loaded is ~ $4e^{-1}$ mbar.

By using a glass microscope slide sample plate, transmission geometry (TG) laser ablation becomes ultimately simple with ionization occurring in vacuum. *Currently the prototype requires the user follow all safety procedures for operating the laser, including laser safety glasses and appropriate sign on doors in accordance with the current edition of ANSI Z136.1 standards*.[61] The most significant changes between vMAI, vMALDI, and vacuum laser desorption (vLDI) are matrices which are volatile, involatile, or no matrix, respectively, as well as the charge states formed and the preferential application. For the results provided here, a 337 nm nitrogen laser was used (Spectra Physics VSL-337ND-S, Mountain View, CA).[26]

Vacuum Matrix Assisted Ionization (vMAI) Method: In vMAI, 0.2-0.5 µL of analyte solution was applied onto the microscope slide, followed with 0.2-0.5 µL of matrix or binary matrix solution. The mixture of matrix:analyte solution was allowed to crystallize for *ca*. 30 seconds on the microscope slide. Droplet spreading outside the diameter of a spacer plate channel diameter was avoided. The microscope slide was then attached to the spacer plate and the assembly inserted into the multi-mode ionization source rails. The sample plate assembly abuts against the previous sample plate and slides in the rail to replace the previous sample plate assembly. Properly designed spacer plates with flat abutting ends make a sufficient seal that the vacuum guage barely deflects during sample plate exchange. A variety of sample plates have been used for vMAI, as previously reported; *e.g.*, metal, Teflon, and glass slides.[24,62]

Vacuum Matrix Assisted Laser Desorption/Ionization (vMALDI) Method: For vMALDI, a 2,5-DHAP matrix stock solution was prepared by dissolving 50 mg of 2,5-DHAP in 1 mL of methanol and matrix:analyte samples were prepared in a **simple, obtaining high throughput without carryover while maintaining** manner similar to the vMAI sample preparation protocol, except using ratio 3:1 *v*/*v* matrix:analyte and dried after application of 0.2–0.5 µL to the glass slide. For CHCA, a saturated solution in ethanol was used. After exposing matrix:analyte sample to the vacuum of the mass spectrometer, for vMALDI, a nitrogen (N_2) laser (337 nm) was used in TG alignment relative to the matrix:analyte sample and the mass spectrometer. The laser beam is focused to *ca.* ~50 µm diameter spot size on the vacuum side of the glass sample plate. With the TG laser arrangement, the laser beam must penetrate through the sample holder and sample to produce a mass spectrum.

Result and Discussion

Ionization of volatile, nonvolatile, low-, and high-mass compounds with excellent sensitivity occurs when specific matrix compounds associated with analyte are exposed to the vacuum of a mass spectrometer. While this vMAI method is exceedingly simple, obtaining high throughput without carryover while maintaining ease of operation has required a rethinking of the ion source used in MS. Probe vMAI sample introduction has desirable attributes of essential elimination of carryover and instrument contamination while achieving high sensitivity, but the mechanics associated with single-probe introduction limits high-throughput. Therefore, we built a prototype plate based source implemented on a Thermo Fisher Scientific Q-Exactive Focus mass spectrometer as shown in **Figure 1** which provides rapid sample analyses and sample plate interchanges at AP while ionization occurs in vacuum. The method of exposing the

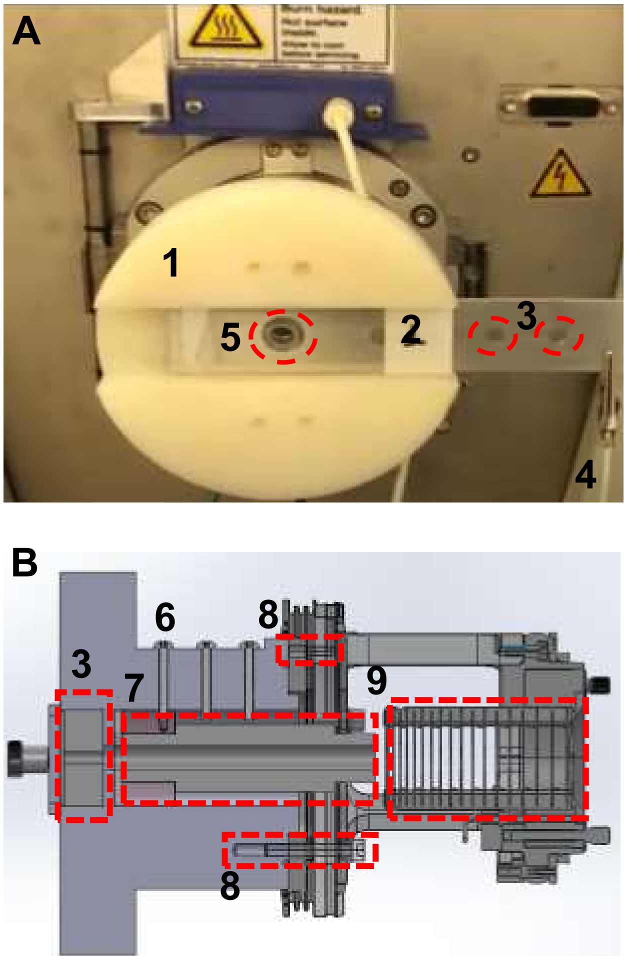

Figure 1: (**A**) A photograph of the prototype multi-mode vacuum ionization source on a Thermo Q-Exactive Focus mass spectrometer; (1) flange, (2) microscope glass slide, (3) a spacer plate with multi sample holes, (4) voltage cable, (5) hole from the spacer plate aligned with the flange opening. (**B**) Cross-section of the multi-mode vacuum ionization source; (3) spacer plate, (6) electrical connector to heated ion guide tube, (7) heated ion guide tube inside the flange, (8) screw connecting the flange to the outer cage containing S-lens (9) S-lens of the Thermo Q-Exactive Focus mass spectrometer.

sample to vacuum and maintaining the vacuum of the mass spectrometer involves use of stacked flat plates which are able to slide one over the other to achieve the desired alignment. Because it is only necessary to expose a single sample to vacuum to obtain its mass spectrum, there is no need for additional pumping, a time consuming vacuum lock, or the cost of manipulating the sample plate in vacuum because all moving parts are accessible from AP. Moving each sample over a channel (hole) in a flange separating AP from the vacuum on the mass spectrometer provides a fast and simple means of sequential ionization and is well suited for high throughput automated analyses. Further, the multi-sample plate assemblies consisting of a sample plate and spacer plate can be interchanged in *ca.* 2 sec. Use of glass microscope slides as sample plates allows TG MALDI under vacuum conditions, but reflective geometry MALDI used with commercial MALDI sources should also be possible.

Using this approach with vMAI, ions are produced from the surface of a respective sample placed under sub-AP until the sample is moved out of the position over the hole that provides access to the vacuum of the mass spectrometer, or the matrix is depleted, whichever occurs first. Depending on the matrix selected, excellent quality mass spectra can be achieved in as little as one or two seconds exposure to vacuum. Typically, for vMAI, the pressure reading on the inlet vacuum gauge of the Q-Exactive Focus is between 0.4 and 1.1 mbar and a voltage of between 60 and 200 V is applied to the metal spacer plate. The voltage applied is dependent on the configuration of the sample and spacer plates but once optimized for any configuration does not need to be changed.

Mass spectra obtained using vMAI on the multimode vacuum ionization source for a variety of compounds, including LSZ, gramicidin S, erythromycin, Al-lad, 1P-LSD, and HCQ were acquired at a rate of *ca.* 1 sample every 4 seconds and detected with good ion abundance (high **e^4** to **e^6**) and little chemical background using 3-NBN as matrix (**Figure 2**). Currently, the speed of analysis with vMAI is limited by initiation of the ionization process once the sample is exposed to sub-AP and to a lesser extent by carryover which may be caused by small bits of matrix popping off the surface and continuing to produce ions until completely sublimed. Analysis times of 3 seconds per sample have been achieved by loading only 0.2 µL of matrix:analyte solution onto the plate. This is not only faster than Agilent's RapidFire,[57] but because ionization occurs directly from biological samples and buffers, and nonvolatile materials remain on the sample plate except the minute amount that is ionized, sample desalting is not necessary to achieve high ion abundance and robust operation, as set out to accomplish relative to previous vacuum-probe devices.[55,57]

Using the prototype vacuum ionization source with TG vMALDI, peptides and drugs contained in complex biological matrices are readily ionized singly charged using typical MALDI matrices. vMALDI-MS uses the sample plate assembly with

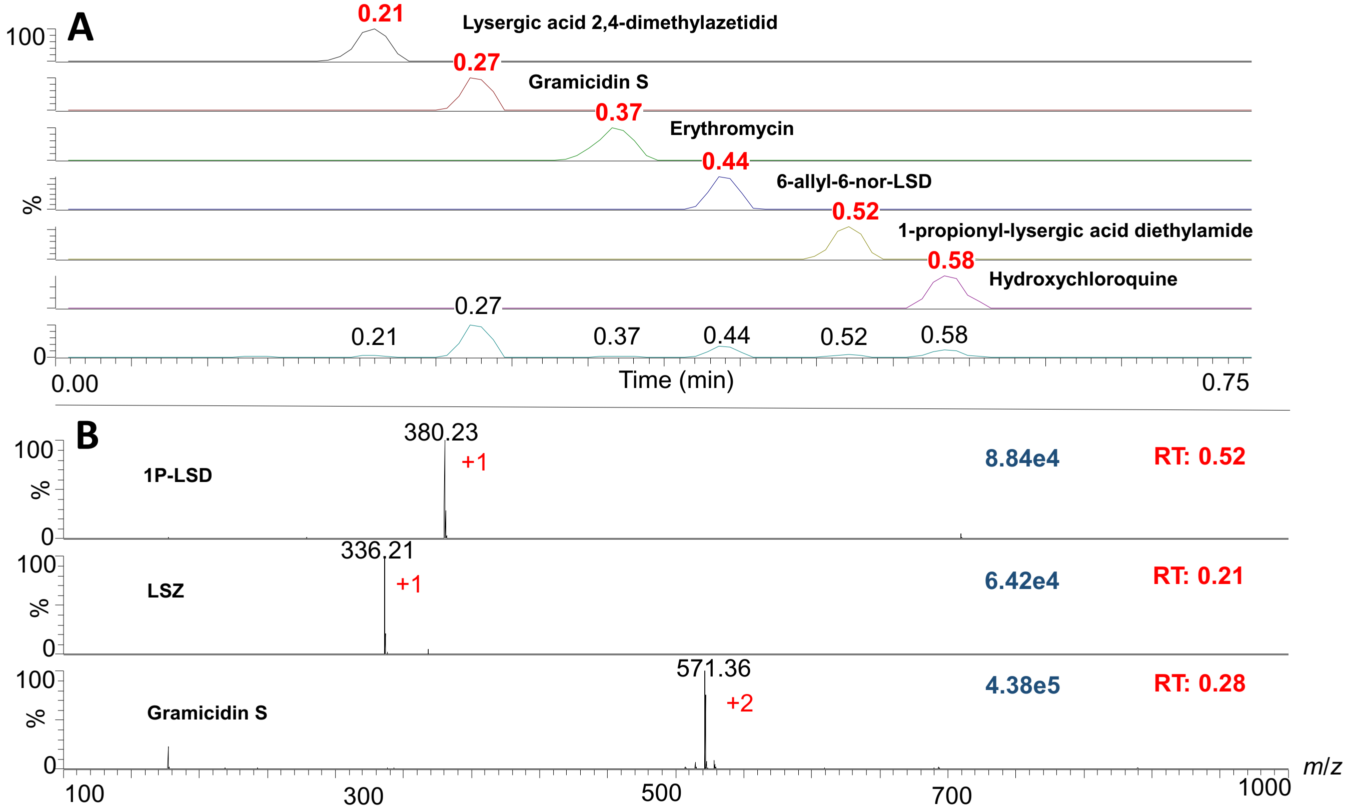

Figure 2: Fast analysis were performed using vMAI at *ca.* 4 seconds per sample. (**A**) Selected ion chronogram of six samples plus total ion chronogram (bottom graph). (**B**) Mass spectra of 1P-LSD (*m/z* 380.23), LSZ (*m/z* 336.21) and gramicidin S (*m/z* 571.36) obtained on a Q-Exactive Focus mass spectrometer.

the spacer plate and glass microscope slide illustrated in **Figure S1** with a nitrogen laser aligned in TG mode. Common MALDI matrices are sufficiently involatile not to sublime/evaporate under vacuum conditions, contrary to vMAI matrices, therefore a laser beam is necessary to initiate ionization. The analyte ions are generated only in the area receiving sufficient laser energy to produce analyte ions. Thus, the vMALDI mode interrogates a small area of the matrix:analyte sample initiated by one or more laser pulses. Moving the point of ablation by either moving the sample plate or the laser beam allows improved sensitivity by summing mass spectra. We hypothesize, based on previous results,[26,28] that with a newer laser having more power and a faster repetition rate, shorter analysis times will be achieved. Importantly, analyte ions are only produced when the laser is fired, so that once the laser beam does not strike the sample, no ions are recorded. This allows acquisition of mass spectra from sequential single samples at *ca.* 1 per second which has only recently been achieved with much more expensive high repetition lasers on high vacuum MALDI-TOF mass spectrometers such as the rapifleX MALDI-TOF by Bruker.[63] An example of vMALDI on the Q-Exactive Focus is shown for acquisition of mass spectra from a blank containing only matrix plus five matrix:drug samples with analytes Al-lad, LSZ, 1P-LSD, gramicidin S, and HCQ, each applied to a glass microscope slide in a 2,5-DHAP matrix solution and dried (**Figure 3**).

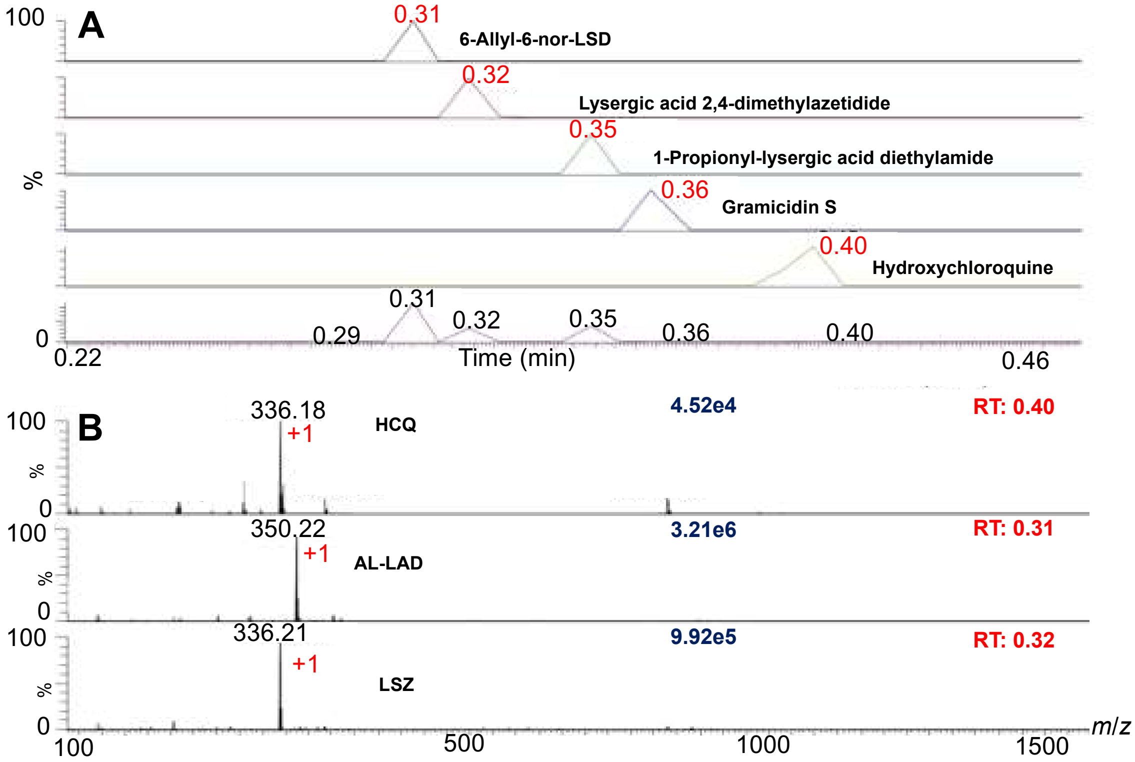

Figure 3: Fast analysis, *ca.* 1 second per sample, were obtained using vMALDI on a Q-Exactive Focus mass spectrometer. (**A**) Selected ion chronogram of five analytes (top to bottom): AL-LAD (1.5 nM), LSZ (1.5 nM), 1P-LSD (1 pM), gramicidin S (1 pM), HCQ (1 pM), and (bottom) TIC of a blank as the first sample at 0.29 minutes. (**B**) Mass spectra of HCQ (*m*/*z* 336.18), AL-LAD (*m*/*z* 350.22) and LSZ (*m*/*z* 336.21).

Using the N_2 laser and TG set-up, individual mass spectra were observed as shown for HCQ, Al-Lad and LSZ in **Figure 3B**. The selected ion chromatogram of the MH^+ ions from all five samples are shown in **Figure 3A**. No carryover was observed from previous samples in any of the mass spectra even with high sample loading of 1.5 nanomole (Al-lad, LSZ), and lower loadings of 1 picomole for the other samples. Contrary to typical MALDI results, no matrix background is observed. Spatial resolution are the same as those previously reported.[64,65]

Synthetic urine samples were spiked with 1P-LSD and HCQ to make 1 and 3 pmol μL^{-1} solutions. The spiked urine solutions were mixed with CHCA matrix solution. The vMALDI mass spectra of two drugs are shown in **Figure 4**. Even though the synthetic urine contains high levels of salt and other compounds, the protonated molecular ions of the analytes were observed well above the background at mass-to-charge (*m*/*z*) 380.23 for 1P-LSD and *m*/*z* 336.18 for HCQ, respectively. Because full acquisition mass spectra are acquired with good analyte ion abundance, it strongly suggests that detection limits will be a few femtomoles in urine which is well below the cutoff level for drug tests.[66]

Because the sample is acquired on a high resolution Orbitrap mass spectrometer, excellent mass accuracy is obtained; 1P-LSD ($C_{23}H_{29}N_3O_2$) with a theoretical *m*/*z* of 380.2333, had a measured value of *m*/*z* 380.2332. Further, collision-induced dissociation (**CID**) can be applied to obtain structural information or more specificity as demonstrated in **Figure S2** for 6-allyl-6-nor-LSD. Thus, high throughput, high sensitivity analyses using MALDI and/or vMAI can readily be obtained on high performance API mass spectrometers.

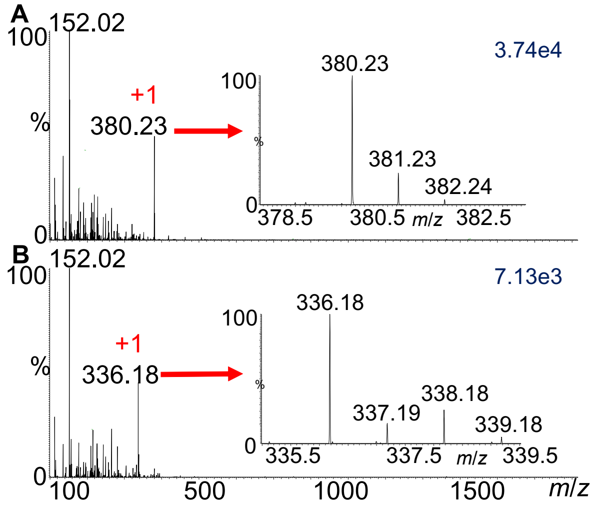

Figure 4: vMALDI direct analysis of urine spiked with two different drugs. (**A**) 1 pmol μL^{-1} 1P-LSD (*m*/*z* 380.23) and (**B**) 3 pmol μL^{-1} HCQ (*m*/*z* 336.18) obtained on a Q-Exactive Focus mass spectrometer.

The multi-mode vacuum ionization source of the apparatus described in **Figures S1** improves the capabilities of a mass spectrometer by fast switching between vMAI and vMALDI as demonstrated in **Movies S1** and **S2**. Importantly, vMAI and vMALDI are methods which produce complementary results. An example from a mixture of ubiquitin and PEG-2000 is shown in **Figure 5**. vMAI was obtained with 3-NBN as matrix, and vMALDI with 2,5-DHAP as matrix. The time to obtain these mass spectra was less than 30 seconds using the manual prototype vacuum source. In other analyses, we have demonstrated acquisition of vMAI and MALDI in <10 sec. Because vMAI with the 3-NBN matrix does not ionize well by

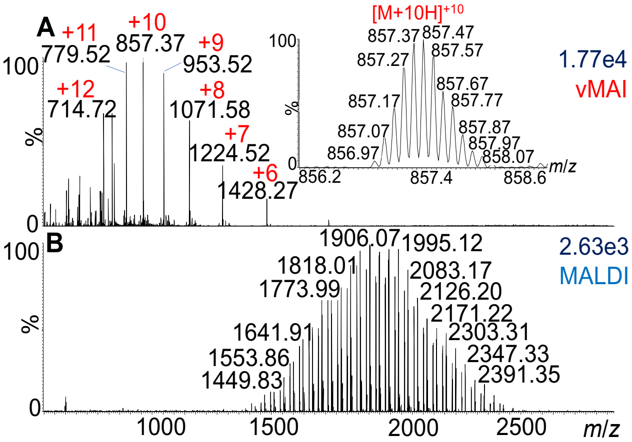

Figure 5: Mass spectra of a mixture of 5 pmol µL^{-1} each of ubiquitin and PEG 2000 polymer using (**A**) vMAI with a 1:1 ratio (*v*:*v*) of 3-NBN and methylbenzoateas binary matrix, and (**B**) vMALDI with DHAP as matrix. With the prototype vacuum source the mass spectra were acquired in less than 30 seconds on a Q-Exactive Focus mass spectrometer.

cation addition, the PEG is not observed, but the ubiquitin provides high ion abundance multiply charged ions. On the other hand, vMALDI does not detect ubiquitin, possibly because the charge states produced are out of the mass range of the instrument, but does produce abundant singly charged ions of PEG.

Another example of the complementary nature of the two ionization methods was obtained from a fungus growing on a strawberry. The same fungus on a wooden toothpick was smeared on a glass plate covering two sample positions. Two microliters of formic acid (70%) was applied over the smear and allowed to dry to directly lyse the samples on the glass plate. After the formic acid dried, 1 µL of a 2,5-DHAP matrix solution was applied to one sample position, and 1 µL of a 3-NBN matrix solution to the other. For the sample spot with the 3-NBN matrix, ionization occurred spontaneously when the sample was exposed to vacuum (**Figure 6A**), and by moving to the sample spot with the 2,5-DHAP matrix, the laser needed to be fired to obtain the mass spectrum (**Figure 6B**). In this case, both methods produced singly charged ions. An *m/z* 200 range window is shown for clarity. Ions common to both methods are bolded in purple, and common to only one method in black. In this mass range, no matrix ions are observed. This result demonstrates the benefit of using two complementary ionization methods for a more complete analysis on the same mass spectrometer. Especially with vMAI, continuous ion current can be obtained over several minutes allowing multiple MS/MS spectra to be acquired using, *e.g.* data dependent MS/MS analyses. Should an ESI mass spectrum be needed, the multi-mode source can be switched with the ESI source in about 30 minutes plus pump down time.

The complementary strengths of vMAI and vMALDI methods using multi-mode vacuum ionization source is also shown analyzing an old protein digest labeled ‘1 pmol/µL BSA trypric digest’ (**Figure S3**). The vMAI mass spectrum is deconvoluted to the singly charged representation for better comparison with MALDI. For these analyses, vMALDI used a 1:1 2,5-DHAP/CHCA matrix and vMAI used 3-NBN. Ions in common to both methods are bolded in purple and unique ions are in black. The combination of the two

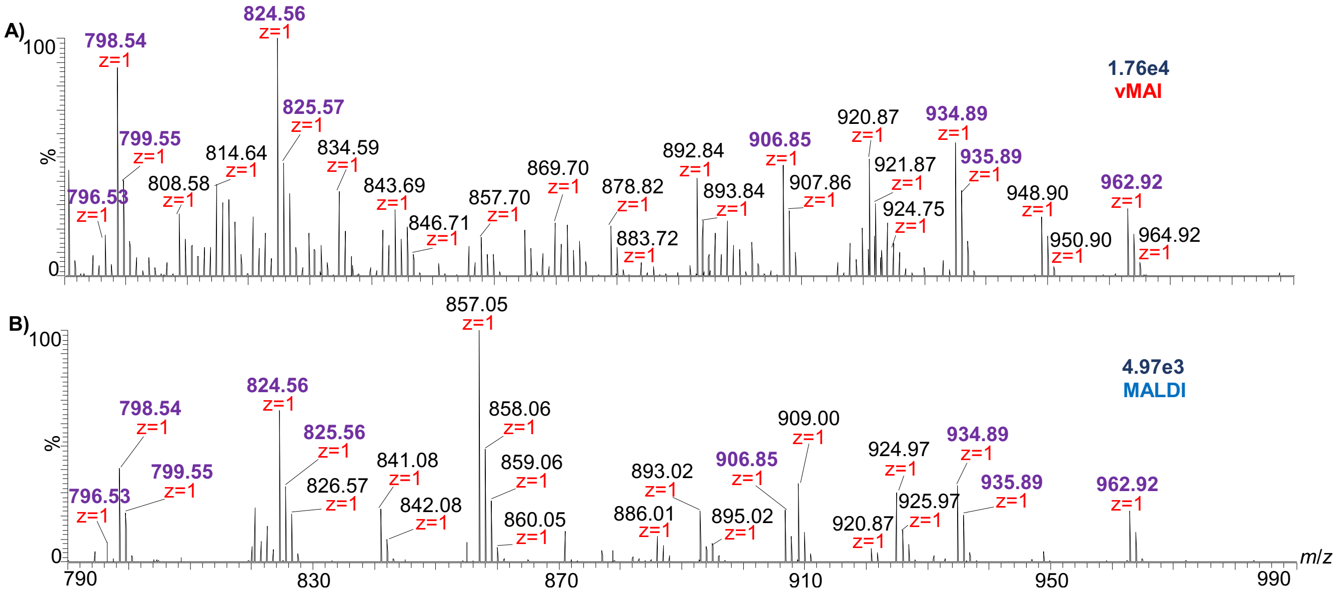

Figure 6: Analysis of a fungus on strawberry using (**A**) vMAI with 3-NBN matrix and (**B**) vMALDI with 2,5-DHAP matrix. Numbers in purple represent the same *m/z* ions observed from both techniques on a Q-Exactive Focus mass spectrometer. The other ions are observed only for one method.

ionization methods provide a more complete information content of the chemical composition of the peptides present in this enzymatically digested protein. Further, the vMALDI mass spectrum produces only singly charged ions, as expected, while the vMAI mass spectrum produces both singly and multiply charged ions, as also expected. Multiply charged ions effectively extends the mass range that is covered by vMAI as seen in the detection of the peptide at *m/z* 4515. This result once again exemplified the advantages of vMAI for mass range limited API mass spectrometers.

Conclusion

The results presented here represent proof-or-principle work with a novel prototype ion source allowing full range mass spectra to be acquired in as little as 1 second per sample at femtomole levels, and providing alternative complementary *vacuum* ionization methods of vMAI and vMALDI. Adjacent sample spots on a multi-sample plate can be acquired by vMAI or vMALDI depending on the matrix used and whether the laser is used or not. All moving parts of the source are accessible from AP for simplicity of operation, but ionization occurs in vacuum for increased sensitivity and robustness. Multiple compounds can be acquired from a single sample plate by either ionization method and sample plates can be interchanged in as little as 2 seconds, thus providing high throughput capabilities once automated. The source also allows for prolonged ionization for multiple MS^n acquisitions, and single samples can be loaded, acquired, and unloaded in less than 10 seconds.[67] The vMAI method produces multiply charged ions without need of a laser allowing mass spectra to be obtained from proteins and other high-mass compounds while vMALDI extends the number of useful matrices and thus compound classes which can be ionized with high sensitivity. The new ion source can be readily interchanged with the commercial ion source in as little as 30 minutes and is potentially adaptable to ultra-high performance or low-cost portable API mass spectrometers. Ionization of the protein ubiquitin, a PEG polymer, drugs in urine, and direct analysis of fungi are demonstrated with comparison between vMAI and vMALDI acquisitions. Further development and optimization is expected to further improve the sensitivity and analysis speed between samples using this multi-mode *vacuum* ionization source. The ionization source has potential to be applied to biological, clinical, biomedical, forensic, agriculture, drugs of abuse, pharmaceuticals, amongst other classes of compounds, as well as for identifying microbes and biomarkers for disease at lower cost, greater simplicity, higher speed, high spatial resolution, and state-of-the-art sensitivity.

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The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript.

ACKNOWLEDGMENT

This material is based in part upon work supported by the National Science Foundation under an NSF Phase II STTR (1556043) including TECP and NSF SBIR Phase I 1913787. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

Supporting Information: Supporting Information (**Figure S1-S3**), the capabilities of a mass spectrometer by fast switching between vMAI and vMALDI as demonstrated **Movie S1** and **Movie S2**.

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