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Desorption sonic spray ionization for (high) voltage-free ambient mass spectrometry

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Sonic spray ionization is shown to create a supersonic cloud of charged droplets able to promote efficient desorption and ionization of drugs directly from the surfaces of commercial drug tablets at ambient conditions. Compared with desorption electrospray ionization (DESI), desorption sonic spray ionization (DeSSI) is advantageous since it uses neither heating nor high voltages at the spray capillary. DeSSI therefore provides a more friendly environment in which to perform ambient mass spectrometry (MS). DeSSI-MS is herein evaluated for the analysis of drug tablets, and found to be, in general, as sensitive as DESI-MS. The (high) voltage-free DeSSI method provides, however, cleaner mass spectra with less abundant solvent cluster ions and with enough abundant analyte signal for tandem mass spectrometry (MS/MS). These features may therefore facilitate the DeSSI-MS detection of low molar mass components or impurities, or both. The higher-velocity supersonic DeSSI spray also facilitates matrix penetration thus providing more homogenous sampling and longer lasting ion signals.

Ambient mass spectrometry, that is, the ionization and mass spectrometric characterization of analytes directly from their natural matrices via a sample preparation-free procedure under atmospheric pressure and at room temperature, is one of the most-welcomed advances in modern mass spectrometry. These unique features also greatly facilitate the on-site application of mass spectrometry. This 'high vacuum-to-real world' transition of mass spectrometry (MS) started in 2004 with the introduction of desorption electrospray ionization (DESI),[1] which was followed by direct analysis in real time (DART),[2] and the analysis of samples at atmospheric pressure (ASAP).[3] In DESI-MS,[4] a spray of electrosprayed charged droplets carried in a supersonic gas stream bombards analytes placed on surfaces. The analytes are believed to be desorbed and ionized mostly by a droplet pick up process.[3] The gaseous ions ultimately generated from DESI are then directed to the atmospheric sampling orifice of the mass spectrometer. Usually, an acidic mixture of methanol and water (1:1) is sprayed at a flow rate of 3–15 $\mu L\,min^{-1}$.

In DESI, high voltages (typically 4 kV) are applied to the capillary tip, and this feature may therefore complicate on-site analysis. As with conventional ESI,[5] sonic spray ionization (SSI)[6] uses polar (typically methanol/water) solutions of the analyte that are sprayed from a fused-silica capillary with a supersonic nebulizing gas flow coaxial to the capillary. SSI is unique, however, since neither heating nor voltage is used for ion formation. Charged droplets and consequently gaseous ions are produced at atmospheric pressure due to statistical (unbalanced) charge distribution during droplet formation in the supersonic pneumatic spray. SSI is therefore gentler than ESI, producing ions of lower internal energy and lower charge states.[7] Electrosonic spray ionization (ESSI) is a variant of SSI, and its main feature is the variable electrostatic potential that can be tuned for most efficient ionization.[8]

It has been reported[4] that the DESI-MS signal intensity does not drop to zero in the absence of an electrospray voltage; hence that the DESI source could also work (although at much lower sensitivity) in the SSI mode, but the features and applicability of desorption SSI (DeSSI) have not yet been addressed (Note: A brief report on low sensitive DeSSI performed using no capillary voltage and its possible application to *in vivo* analysis can be found in Ref. 4.) We now report, for drugs in tablets, that DeSSI is able to provide an adequate supersonic cloud of charged droplets for the efficient desorption and ionization of analytes directly from tablet surfaces providing therefore an alternative[9] and more friendly (high) voltage-free environment for ambient MS. DeSSI is also found to provide more homogenous sampling and cleaner, more stable and longer lasting mass spectra, with ion abundances sufficient for tandem mass spectrometric (MS/MS) investigation.

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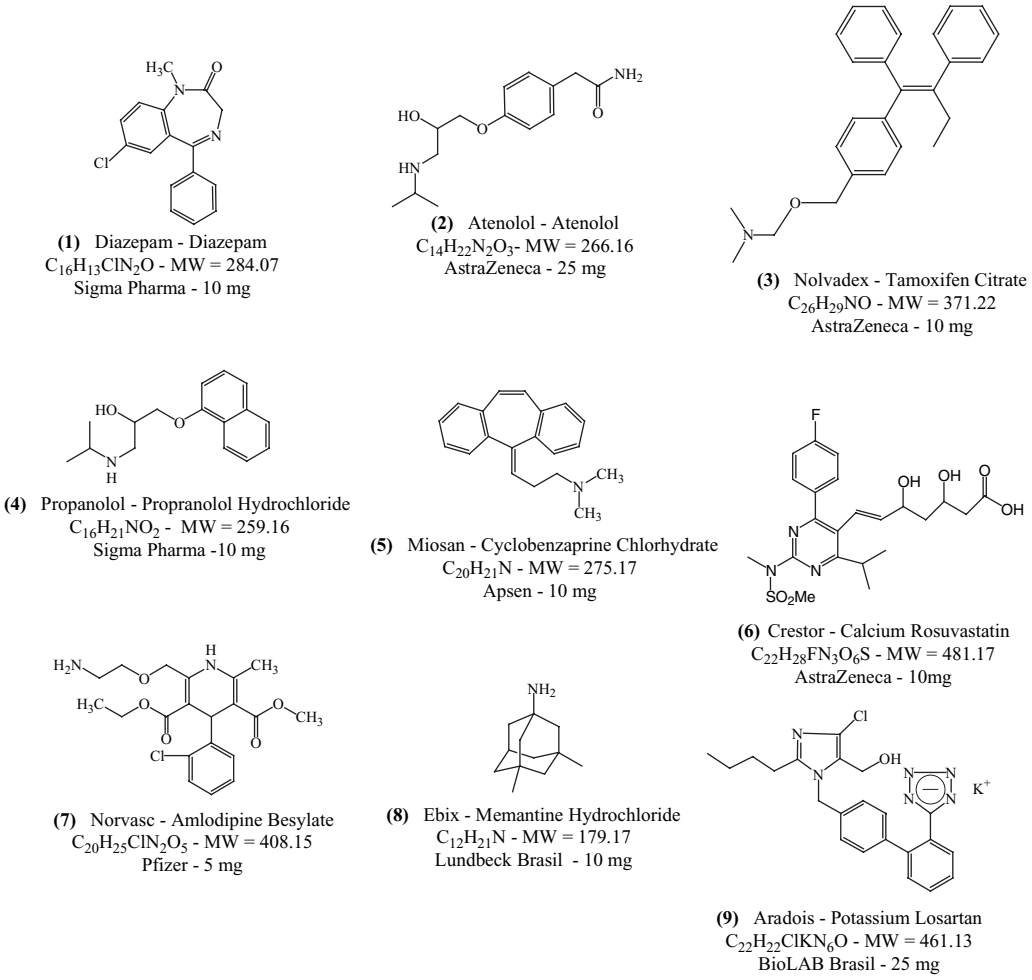

Scheme 1. Structure, commercial name, major component, molar mass, manufacturer and the amount of drug per tablet for the set of drug tablets investigated.

EXPERIMENTAL

Chemicals

Formic acid and HPLC-grade methanol were purchased from Merck SA (Rio de Janeiro, Brazil) and used without further purification. Deionized water was obtained from a MilliQ (Millipore, Billerica, MA. USA) purification unit. Scheme 1 summarizes data from the set of nine commercial drug tablets selected for this study.

Mass spectrometry

Experiments were performed on a Q-Trap® hybrid triple-quadrupole mass spectrometer (Applied Biosystems do Brasil, São Paulo, Brazil) using a homemade SSI source similar to that described by Cooks and coworkers[7] and mounted on a commercial nano-ESI source (Applied Biosystem do Brasil), which is described in detail elsewhere.[10] The mass spectrometer was operated in the positive ion mode. Tablet **3** was used as a reference tablet to establish the best operating conditions for DeSSI, i.e. flow rate of the 1:1 acidic (0.01% formic acid) water/methanol solution of 20 μL min^{-1}, nebulizing gas backpressure of ca. 30 bar, curtain gas pressure of 5 bar, declustering potential of 100 V, tip-tablet and tip-entrance distances of ca. 2 mm, and capillary-tablet-entrance angle of ca. 30°. Tandem mass spectrometric experiments were performed using the product ion scan mode via Q1 selection of the desired precursor ion, q2 collision-induced dissociation (CID) of that ion with nitrogen, with detection of the CID product ions in Q3. The collision energy ranged from 10 to 40 eV, as required to obtain a reasonable product ion spectrum from the precursor ion.

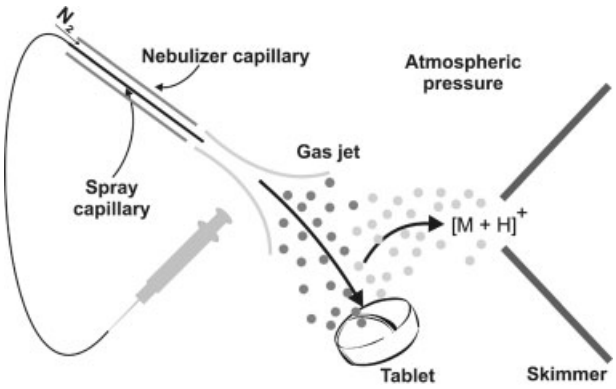

Figure 1. Schematic of desorption sonic spray ionization (DeSSI) for (high) voltage-free ambient mass spectrometry as applied to drugs in tablets.

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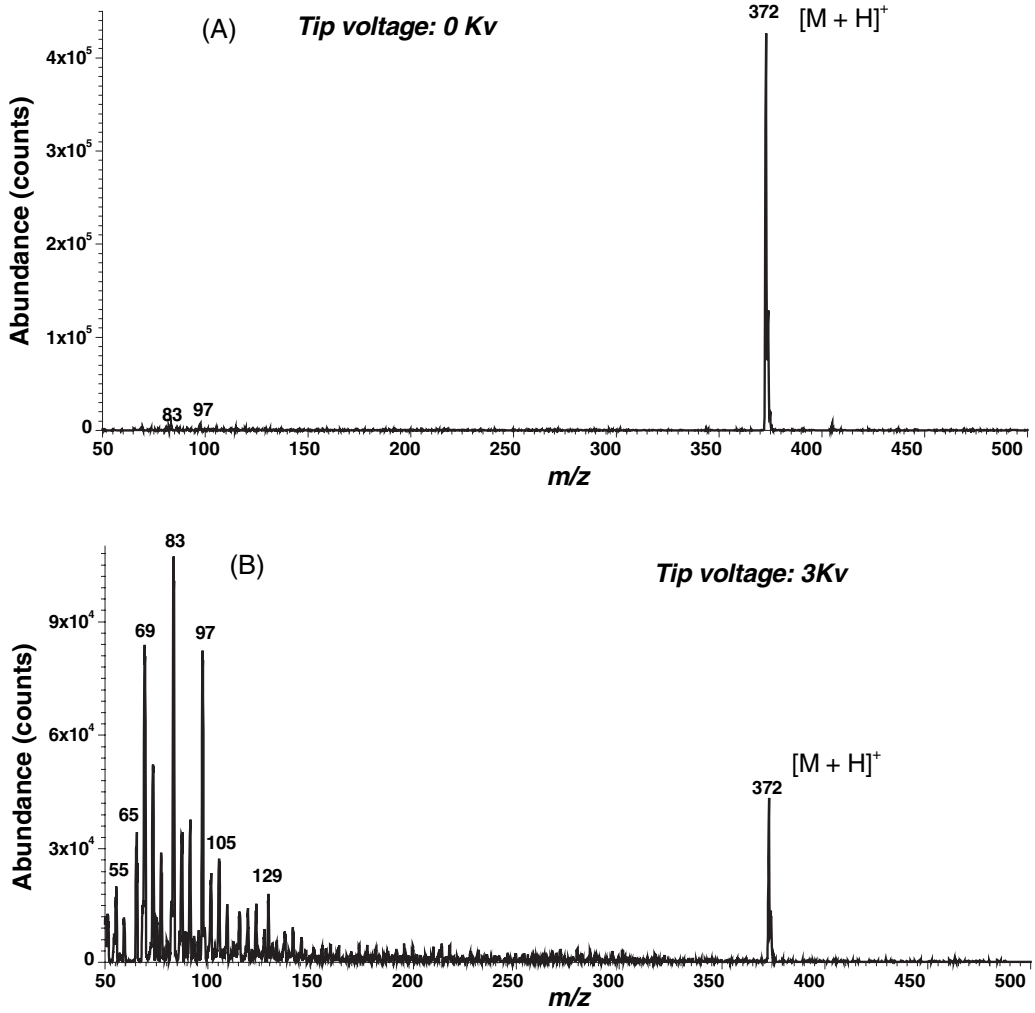

Figure 2. (A) DeSSI and (B) 3 keV DESI mass spectra in the positive ion mode for tablet **3**. The ion of *m/z* 372 is protonated tamoxifen (Scheme 1) whereas the abundant low *m/z* ions in (B) are solvent (water/methanol) cluster ions, see text.

RESULTS AND DISCUSSION

DeSSI versus DESI

Figure 1 displays a schematic of the DeSSI process. After optimizing the DeSSI-MS response for tablet **3** (see Experimental section), all the tablets were run under these optimized conditions. A comparison was made of the ionization yields by measuring the abundances of the protonated analyte molecules with and without the application of variable voltages at the tip of the solution syringe. The tablets were placed on a holder mounted on the SSI source and were in some cases scraped to expose an uncoated surface prior to analysis. Typically, a few seconds of exposure were required to generate a representative mass spectrum, where signal variation for optimized DeSSI conditions was typically 20%. For both DESI and DeSSI, we note that the mass spectral signal intensities vary considerably with operating conditions. Thus, great care has to be taken to find the best operating conditions for each technique.

Figure 2 shows the DeSSI and DESI mass spectra for tablet **3**. Figure 3 displays the absolute intensities of the protonated analytes for tablets **3**, **4** and **5**, as well as for a

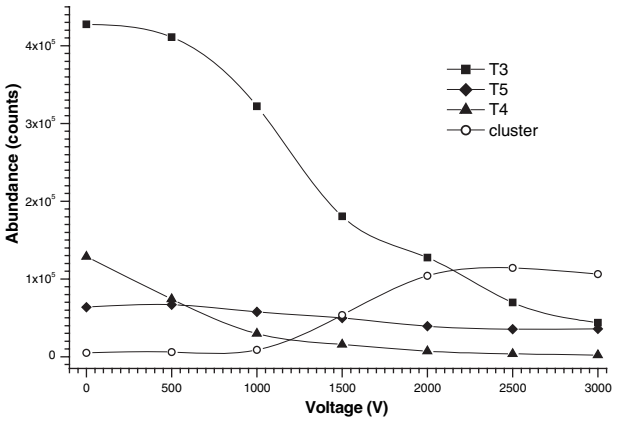

Figure 3. Absolute abundances (counts) in the DeSSI (0 V) or DESI (variable voltages) mass spectrum as a function of the capillary voltage for the protonated analytes of tablets **3–5** (T3–T5) as well as a reference solvent cluster ion $[2CH_3OH + H_2O]^+$ of *m/z* 83. Average values from triplicate measurements with a signal deviation of ca. 10–20%.

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typical solvent cluster ion of m/z 83, i.e. $[2CH_3OH + H_2O + H]^+$, using voltages ranging from 0 to 3 kV. As a rule, using the best source conditions for DeSSI, the higher the voltage the higher the abundance of solvent cluster ions and the lower the abundance of the protonated analyte molecule (Fig. 3). Major solvent clusters (and their probable compositions) observed under these conditions were of m/z 55 $[3H_2O + H]^+$, m/z 65 $[2CH_3OH + H]^+$, m/z 69 $[CH_3OH + 2H_2O + H]^+$, m/z 83 $[2CH_3OH + H_2O + H]^+$, m/z 97 $[3CH_3OH + H]^+$, m/z 105 $[CH_3OH + 4H_2O + H]^+$ and m/z 129 $[4CH_3OH + H]^+$. Compared with ESI, SSI is known to produce droplets with a lower degree of charging.[5] We therefore interpret the data from Fig. 3 as an indication that under DESI with increasing voltages, a too dense cloud of charged droplets with a too high concentration of solvent cluster ions is formed, which ionizes the analyte efficiently but produces a too dense cloud of gaseous solvent clusters ions in which the protonated analyte molecule is a minor component. The major solvent cluster ions compete therefore with the protonated analyte molecule during MS transmission and detection. DeSSI provides, however, a less dense cloud of charged droplets with a lower, non-excessive concentration of solvent cluster ions that produces a beam of gaseous ions in which the protonated analyte molecule dominates.

To compare the performance of DeSSI with that of DESI more adequately, we tried to tune source conditions (mainly nebulizing and declustering gas pressures and temperatures) in order to favor either DeSSI or DESI for each analyte.[5] In general, the optimal DeSSI nebulizing pressure was 2–5 times higher than that used in DESI, whereas optimal DESI voltages ranged from 4 to 5.5 kV. Figure 4 presents the absolute intensities of the protonated analyte molecules under these optimized DESI and DeSSI conditions. These intensities vary greatly according to the nature of the drug and the physicochemical properties of the tablet.[11] Overall, however, similar performances are observed for both DESI and DeSSI. The DeSSI mass spectrum is nevertheless always cleaner owing to much lower abundances of solvent cluster ions. This interesting feature of DeSSI may therefore facilitate the mass spectrometric identification of low molar mass

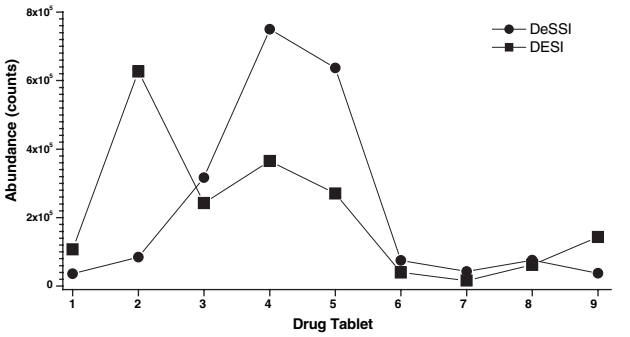

Figure 4. Absolute abundances for the protonated analytes in the DeSSI and DESI mass spectra of drug tablets **1**–**9** (See Scheme 1).

tablet components or contaminants, or both. The high-velocity supersonic spray normally required for best DeSSI performance also seems to facilitate deeper tablet penetration, thus providing more homogenous sampling. A more stable and long-lasting ion signal was also observed for DeSSI than for DESI.

DeSSI also seems to work efficiently in the negative ion mode, as illustrated in Fig. 5 for the DeSSI mass spectrum of a commercial aspirin tablet. Although we have not investigated the nature of all detected ions (that of m/z 137 is probably a fragment formed by ketene loss), the abundant ion of m/z 179 shows that the target analyte is clearly detected as its deprotonated molecule.

As a representative example, Fig. 6 shows the DeSSI tandem mass spectrum of protonated amlopidine of m/z 409 from tablet **7**. This DeSSI-MS/MS spectrum clearly illustrates that, although the sensitivity of DeSSI-MS may sometimes be quite low (as for tablet **7**, see Fig. 3), the abundance of the protonated analyte molecule was nevertheless always sufficient for the acquisition of its DeSSI tandem mass spectrum with adequate signal-to-noise ratio for structural investigation.

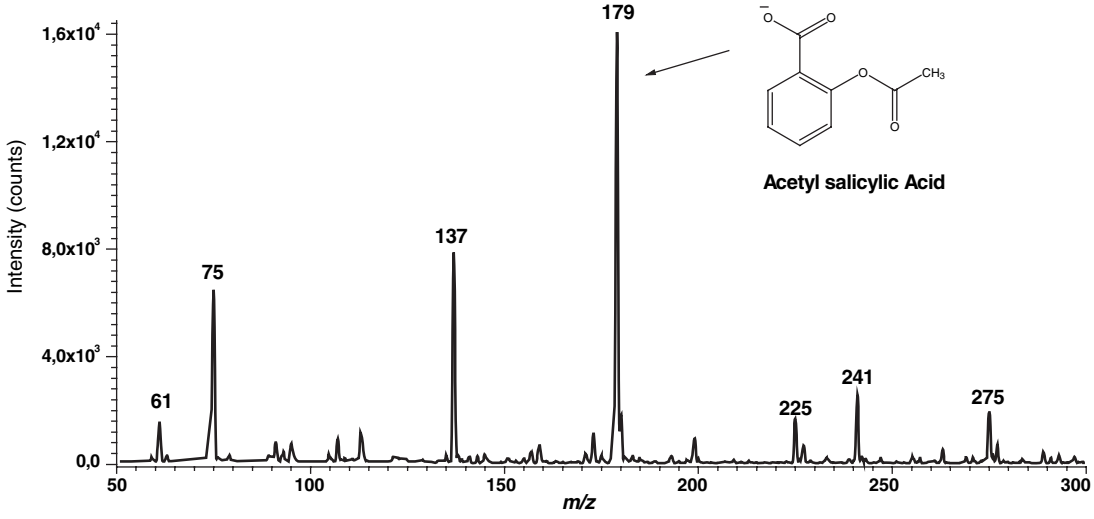

Figure 5. The negative ion DeSSI mass spectrum of a commercial aspirin tablet.

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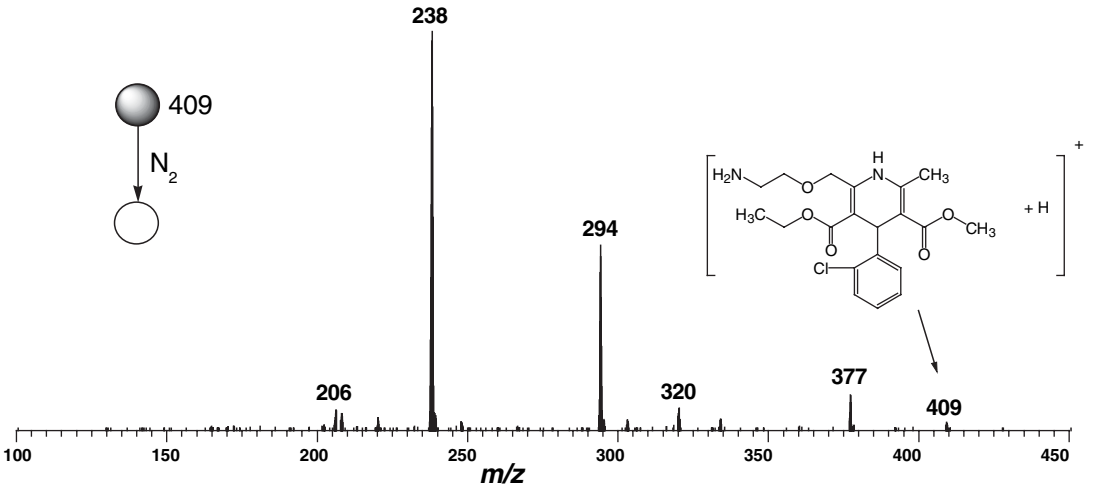

Figure 6. The DeSSI tandem mass spectrum of protonated amlopidine of *m/z* 409 from tablet **7**.

CONCLUSIONS

As demonstrated herein for drugs in tablets, desorption sonic spray ionization (DeSSI) is a convenient means of creating a suitably dense cloud of charged (ion-carrying) droplets for ionization at ambient conditions of analytes placed on surfaces. Compared with DESI, the simpler and gentler DeSSI technique may be advantageous in several cases as it avoids the use of high voltages which are applied to the spray capillary under DESI-MS, thus providing a more friendly (high) voltage-free environment in which to perform ambient ionization. This feature may be important, for instance, when using DeSSI for *in vivo* applications of ambient MS. *In situ* tissue analysis during surgical procedures seems to be a case where the application of DeSSI-MS would be beneficial. The DeSSI mass spectrum is also consistently cleaner since it contains less abundant solvent cluster ions. This interesting feature may facilitate the detection of low molar mass components or impurities, or both. The higher-velocity supersonic spray of DeSSI also facilitates deeper matrix penetration, thus providing more homogenous sampling and a longer lasting ion signal. As in DESI, ion-carrying droplets are used in DeSSI. Therefore, ambient ion/molecule reactions could also be tested via reactive DeSSI[12] to gain sensitivity and/or selectivity, or both. We are currently exploring the use of DeSSI and reactive DeSSI for the mass spectrometric investigation of a variety of analytes, surfaces and matrices, with promising results.

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