Analysis of Pharmaceutical Compounds from Glass, Fabric, Steel, and Wood Surfaces at Atmospheric Pressure Using Spatially Resolved, Nonresonant Femtosecond Laser Vaporization Electrospray Mass Spectrometry

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Laser electrospray mass spectrometry (LEMS) is demonstrated for pharmaceutical samples at atmospheric pressure. A nonresonant, femtosecond duration laser pulse vaporizes native samples at atmospheric pressure into an electrospray plume for ionization with subsequent transfer into a time-of-flight mass spectrometer. The active ingredients in pharmaceutical tablets were detected in the presence of binders and fillers in intact formulations using LEMS. Mass spectra were also obtained for microgram amounts of the pharmaceutical compounds loratadine, oxycodone, and atenolol deposited on glass, wood, steel, and polyester fabric. The neutral capture efficiency by the electrospray plume for nonresonant laser vaporization of oxycodone and atenolol desorbed from steel is 2.4% ± 1.5% and 0.25% ± 0.18%, respectively. LEMS imaging of the spatial distribution of an oxycodone spot on a metal slide with resolution of 250 μm is also presented.

Forensic detection and analysis of narcotics and pharmaceuticals currently employ methods that include high performance liquid chromatography (HPLC), gas chromatography (GC), thin layer chromatography (TLC), UV spectrophotometry, and electrophoresis. In the traditional forensics approach, the substance of interest must be transferred from the native material and/or surface via a swab or elution prior to chemical analysis. Although the current methods for drug analysis work very well for separating, identifying, and quantifying compounds, they remain time-consuming, require substantial sample preparation, and are often not suited to trace analysis.

Direct analysis of a contaminated surface eliminates the use of swabs or solvent elution for sample collection facilitating a higher signal-to-noise ratio (S/N), decreasing the probability for a false negative result. Several new techniques permit the direct detection of trace quantities from native surfaces and complex matrices. One such detection method is desorption electrospray ionization (DESI), a technique that uses electrospray generated droplets directed at a surface to analyze native sample under ambient conditions. DESI has been used to detect trace amounts of explosives on paper, cloth, brick, metal, and skin and the active ingredients of several drug formulations.

The use of resonant laser desorption for ambient mass spectrometry is a rapidly growing field of research that is amenable to direct analysis of samples under certain circumstances. Resonant absorption occurs when the spacing between energy levels (vibrational or electronic) is equal to the energy of one photon, \( h\omega \), where \( h \) is Planck’s constant divided by \( 2\pi \) and \( \omega \) is the angular frequency of the photon. For typical laser desorption mass spectroscopy, a one-photon absorption process is active and, therefore, laser intensities are on the order of \( 10^6 \) W cm\(^{-2} \). Laser-assisted electrospray ionization, LAESI, uses a resonant nanosecond infrared laser where the wavelength of the laser is tuned to be resonant with the OH vibration in water at 2.940 μm. Once excited, the water molecules undergo a phase explosion to eject materials into the gas phase. Experiments performed using water-rich samples include the detection of the antihistamine fexofenadine in urine and blood. Plant tissue has also been analyzed using LAESI. Matrix-assisted laser desorption electrospray ionization (MALDESI) uses a laser in resonance with an electronic transition in the matrix molecules. The matrix molecules are cocry stallized with the sample of interest, to enable laser desorption of the sample. MALDESI has been used to analyze peptides and proteins ranging in mass from 1 to 8.6 kDa. Both of these techniques have sample requirements for successful analysis. LAESI requires the sample to be water rich or to be sprayed with water before analysis, while MALDESI requires a matrix to be deposited onto a surface before analysis can be initiated. MALDESI also requires that the analyte be soluble in water.

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polar matrices. Finally, in both methods, a laser of a specific wavelength is required to induce the necessary resonant transition to couple the laser light into the analyte system.

Electrospray laser desorption ionization (ELDI) is another ambient mass spectrometry technique that is amenable to direct sample analysis in certain cases. ELDI employs a nanosecond UV laser to desorb sample prior to electrospray ionization. At least one component of the sample or substrate must resonantly absorb the UV radiation to induce desorption. Dried biological fluids such as tears, saliva, blood, and serum\(^{19}\) have been analyzed using this technique. Nanosecond ELDI has also been applied to drug tablets, porcine brain tissue, and milk, as well as to the surface of a compact disk.\(^{11}\) Separations of dyes, amines, and drug tablet extracts (using thin layer chromatography) have also been subjected to ELDI analysis.\(^{12,13}\)

Femtosecond laser sources have been receiving increasing attention as an ionization source for gas phase molecules.\(^{14}\) The coupling mechanism between an intense femtosecond laser and a molecule is an active area of investigation,\(^{15–17}\) and the ultrafast excitation results in qualitatively different fragmentation pathways in comparison with nanosecond excitation.\(^{17,18}\) In gas phase nonresonant laser ionization experiments, nanosecond laser pulses cause more fragmentation in comparison with femtosecond laser pulses at comparable intensities. This is due to the prevalence of the ladder switching mechanism and energy deposition into nuclear modes of the molecule in the nanosecond ionization experiment.\(^{19}\) The use of shorter duration laser pulses typically increases the amount of parent relative to fragment ions,\(^{20}\) which is advantageous in the analysis of complex samples where peak assignment in congested mass spectra can be arduous. Here, we apply similar nonresonant ultrafast excitation to the vaporization of nonvolatile molecules from the condensed phase into the gas phase.

The use of nonresonant laser vaporization of biological molecules for mass spectrometry was recently reported.\(^{21}\) The new method employs intense femtosecond duration laser pulses to transfer molecules from the solid state into the gas phase followed by electrospray ionization to enable mass spectral analysis of the vaporized samples at atmospheric pressure. Nonresonant absorption occurs when the spacing between the ground and pertinent excited energy levels is not equal to \(\hbar \omega\). Nonresonant absorption occurs when two or more photons are absorbed (through virtual states) and typically requires high laser intensity, \(>10^8\) W cm\(^{-2}\). In the case of femtosecond nonresonant excitation, the laser will, in principle, couple into all molecules to induce vaporization. This means that sample preparation (elution, mixing with matrix, and choosing samples with a particular absorption spectrum) is eliminated. Two implementations of this method have been demonstrated previously: femtosecond electrospray laser desorption ionization (fs-ELDI) and femtosecond matrix-assisted laser desorption electrospray ionization (fs-MALDESI).\(^{21}\) In these investigations, atmospheric pressure vaporization was demonstrated for a series of pure and matrix-solvated biomolecules with mass up to \(m/z=1355\) (vitamin B12, cyanocobalamin). In these experiments, postvaporization ionization occurred in the electrospray plume. The general class of methods combining nonresonant femtosecond laser vaporization with electrospray ionization mass spectrometry may be called laser electrospray mass spectrometry (LEMS).

We investigate here the direct analysis of pharmaceutical active ingredients in tablet and pure form to demonstrate that LEMS is capable of analyzing complex samples in the presence of commercial binders and fillers without sample preparation. LEMS analysis of molecules on substrates including glass, steel, fabric, and wood demonstrates the interrogation of trace samples on complex, multifunctional materials. We demonstrate that a spatially resolved mass spectral image of oxycodone deposited on stainless steel can also be measured using LEMS. Finally, we determine the neutral capture efficiency of LEMS for the molecules atenolol and oxycodone adsorbed on a steel surface using calibrated electrospray ionization experiments.

**EXPERIMENTAL SECTION**

**Sample Preparation.** The three pharmaceutical drugs used in this study are loratadine, oxycodone, and atenolol. Claritin, an antihistamine, is formulated as a solid tablet containing 10 mg of the active ingredient loratadine [ethyl 4-(8-chloro-5,6-dihydro-11H-benzo [5,6] cyclohepta [1,2-b] pyridin-11-ylidine)-1-piperidinecarboxylate]. Oxycodone (4,5a-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one) is the active ingredient in Oxycontin and Percocet and was obtained in the powdered base form.\(^{22}\) Atenolol is a \(\beta\)-blocker formulated as a solid pill containing 50 mg of atenolol ((RS)-2-4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl)acetamide). To investigate the detection of trace amounts of the active drugs on various surfaces, a Claritin pill was dissolved in 1:1 (v:v) water/acetonitrile to obtain a concentration of \(2.6 \times 10^{-4}\) M loratadine. A \(1.3 \times 10^{-4}\) M solution of oxycodone was prepared by diluting 4.2 mg of oxycodone in 1:1 (v:v) water/acetonitrile. A solution of 1.7 mg of pure atenolol (Spectrum Chemical, Gardena, CA) and 1:1 (v:v) water (Fisher Scientific, Fair Lawn, NJ)/acetonitrile (Fisher Scientific, Fair Lawn, NJ) was prepared at a concentration of \(1.3 \times 10^{-4}\) M, and a 50 \(\mu\)L aliquot was placed on various surfaces including stainless steel, glass (Fisher Scientific, plain microscope slides #12549, Pittsburgh, PA), polyester fabric (purchased from a local fabric store) and wood (Puritan Medical Product Company, standard tongue depressors, 704 Hospital, Guilford, MA). The
glass and steel plates were untreated but were cleaned with methanol and water before sample deposition. The solutions were deposited using a micropipet over an area of 10–160 mm², depending on the sample and surface material. The wood substrate dispersed the solution over a larger area than glass and steel, presumably due to hydrophilic interaction with the solvent. The solution deposited onto the glass and steel substrates tended to bead and dry in a smaller area. The sample plate holder was translated using an XY stepper motor (Zaber Technologies Inc., Vancouver, British Columbia, Canada) driven stage during the data acquisition to ensure fresh sample was available for each laser vaporization event.

The samples employed in the neutral capture efficiency experiment were pure atenolol and oxycodone having concentrations of $1.3 \times 10^{-4}$ M. A 50 µL aliquot of each dilution was placed on steel sample plates (12–60 mm²) for LEMS analysis.

**Mass Spectrometry.** The mass spectrometry apparatus combines nonresonant laser vaporization with an ESI source for ionizing and transferring vaporized sample into a vacuum chamber where pulsed deflection orthogonal time-of-flight (o-TOF) mass spectrometry was performed, with resolving power $(m/Δm)$ of ~300. The ESI source (Analytica of Branford, Inc., Branford, CT) was operated in positive ion mode and consisted of a needle, dielectric capillary, skimmer, and a hexapole system. The needle was maintained at ground while the voltage on the inlet capillary was adjusted until a stable ion current/signal was established, typically around $-5300$ V. The acidified electrospray solvent (1% acetic acid, 1:1 (v:v) acetonitrile/water) was pumped through the needle at a flow rate of 2 µL/min. The electrospray plume was dried by counter current nitrogen gas at 180 °C before entering the dielectric capillary. A more detailed description of the apparatus has been reported elsewhere.  

**Vaporization and Ionization Apparatus.** A Ti:sapphire laser system (oscillator: Coherent Inc., Santa Clara, CA; operating at 10 Hz, 800 nm central wavelength, 400 µJ per pulse with 70 fs pulse duration, was used to vaporize the samples. Figure S1 in Supporting Information shows a schematic of the vaporization and ionization apparatus. The vaporization beam was focused to a spot size of 295 µm in diameter for steel surfaces and 190 µm in diameter for glass, cloth, and wood surfaces with an incident angle of 45° with respect to the sample surface. For the LEMS spatial imaging experiment, the laser was focused to a spot size of 250 µm to increase the spatial resolution. The laser intensity at the surface was $\sim 10^{13}$ W cm⁻². The steel sample holder was biased to $-2.0$ kV to compensate for distortion of the electric field between the needle and capillary caused by the introduction of the sample stage. The vaporized sample was ejected perpendicular to the electrospray plume. The electrospray plume serves to capture, ionize, and transport the ions through an inlet capillary followed by three regions of differential pumping (1.2, $\sim 10^{-3}$, and $\sim 10^{-5}$ Torr) leading to the high vacuum region ($\sim 10^{-7}$ Torr) for mass analysis. The inlet capillary is an 18 cm long dielectric glass capillary (6.4 mm o.d., 500 µm i.d.) coated with metal at both ends. An ESI solvent background was acquired before each LEMS measurement to enable subtraction of solvent-related peaks. When vaporized, the sample molecules compete for charge with the electrospray solvent cluster. Thus, the solvent ion intensity distribution is modulated (both positive and negative) when the laser is present. The negative and positive features in the mass spectrum presented result from subtraction of the ESI background spectrum from the sample spectrum (which has the modulated solvent ion intensity distribution). Mass spectra were averaged for 5 s (50 laser shots).

**Safety Consideration:** Appropriate laser eye protection was worn by all personnel and high voltage area was enclosed in plexiglass to prevent accidental contact with the biased electrodes.

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<th>RESULTS AND DISCUSSION</th>
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**Femtosecond LEMS of Claritin and Atenolol Pills.** To determine the capability of LEMS to directly analyze complex mixtures, a pharmaceutical tablet formulation containing binders and fillers was analyzed. As a first experiment, a Claritin tablet was placed directly onto the sample plate holder and was subjected to LEMS analysis. The mass spectrum of the intact tablet is shown in Figure 1a. The protonated parent ion of loratadine is observed with $m/z = 383$ and 385 in the mass spectrum of the Claritin tablet. While not fully resolved, the 3:1 peak intensity ratio is consistent with the expected isotope distribution for the chlorine atom in loratadine. Other peaks observed in the spectrum include fragments of loratadine ions and inactive ingredients (magnesium stearate and lactose monohydrate). The peaks at $m/z = 253, 267,$ and 281 result from the $\text{C}_8\text{H}_2\text{O}_3$ (n = 1) loss from magnesium stearate ($m/z = 309$), this ion results from the loss of one of the stearate chains). Lactose monohydrate is also present at $m/z = 361$. In loratadine, the three fragment peaks are tentatively assigned to the ethyl-1-piperidinecarboxylate fragment at $m/z = 155$ and the isotopic peaks of the 8-chloro-6, 11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridine fragment at $m/z = 227$ and 229. Other peaks observed in the spectrum such as $[\text{M}+\text{H}+\text{OCH}_2\text{CH}_3]^+$ at $m/z = 337$ and 339 and $[\text{M}+\text{H}+\text{Cl}]^+$ at $m/z = 349$ are consistent with the literature.  

This experiment establishes the capability of LEMS to laser
atenolol at the ether bond to produce \([C\text{ }6H_{14}NO]^{+}\). Detection experiments, rather the features merge to a peak at 3234 for protonated loratadine and atenolol molecules in Figure 1b. The protonated atenolol, \([M+H]^{+}\), is observed with \(m/z = 289\) in the spectrum. The peak at \(m/z = 116\) is consistent with fragmentation of atenolol at the ether bond to produce \([C_{6}H_{13}NO]^{+}\). The detection of protonated loratadine and atenolol molecules demonstrates that LEMS can be used for rapid discrimination of drugs in tablet form without further sample preparation or matrix deposition.

**LEMS of Trace Amounts of Claritin on Different Surfaces.** There is an increasing need to rapidly identify trace molecules, including pharmaceuticals, on various surfaces for forensic analysis. To investigate the use of LEMS as a means to detect trace amounts of pharmaceuticals on various surfaces, 50 \(\mu\)L of the 2.6 \(\times\) \(10^{-4}\) M Claritin solution was deposited on wood, steel, glass, and fabric. The total sample applied to each surface was 1.6 \(\mu\)g, covering an area of 60–160 mm\(^2\). The Claritin sample dries in a larger, more uniform area than the atenolol or oxycodone, presumably due to the binders and fillers present. This even coverage provides a more stable ion signal in comparison with pure compounds. Approximately 750 pmol of Claritin was vaporized during the 50 laser shot acquisition. Figure 2 displays the mass spectrum measured for protonated loratadine on wood, steel, and glass. In comparison with the other pharmaceutical molecules investigated, Claritin had the highest signal intensity on wood, Figure 2a. The excess of binders and fillers present in Claritin presumably modulates surface effects and penetration into the wood substrate. The isotopic features of loratadine at \(m/z = 383\) and 385 are not fully resolved in the trace detection experiments, rather the features merge to a peak at \(m/z = 384\). The parent ion can be seen on both the metal and glass surfaces, Figure 2b,c. Next, Claritin was deposited on the red, black, and white polyester fabrics and subjected to LEMS analysis. Loratadine was not detected consistently on any of the fabrics, although \(\sim10\%\) of the laser shots revealed signal on the white fabric. Unlike atenolol and oxycodone, loratadine was obtained by dissolving a Claritin tablet into solution and was the only pharmaceutical that was not used in its pure form. The lower signal intensity on fabric may also be due to the presence of pores, larger in size than those found in wood, allowing the sample to permeate into the fabric substrate. The vaporization of loratadine from the wood surface demonstrates a new release technology for substances adhered to shipping materials. This is significant since the increase of illegal transport of weapons and drugs on cargo ships requires the development of systems that are able to detect signature molecules on multiple surfaces including shipping crates and luggage.\(^{25}\)

**LEMS of Trace Amounts of Oxycodone on Different Surfaces.** Laser vaporization of pure pharmaceutical molecules was investigated from surfaces including wood, steel, glass, and fabric. A 50 \(\mu\)L aliquot of \(10^{-4}\) M oxycodone solution was deposited on to 10–120 mm\(^2\) of each surface and allowed to dry. This distributed 5 nmol (1.6 \(\mu\)g) of oxycodone over the area, and approximately 650 pmol was vaporized during the acquisition, as estimated from the surface coverage and the number of laser shots. Figure 3a–c shows the mass spectrum acquired after 50 laser shots on wood, steel, and glass, respectively. Oxycodone (\([M+H]^{+}, m/z = 316\) has the highest signal intensity on the steel surface, followed by glass and then wood. The peak at \(m/z = 298\) is consistent with the loss of water from the protonated parent ion. The sodium adduct of oxycodone, \([M+Na]^{+}\) at \(m/z = 338\), is detected when the molecule is vaporized from the steel and glass surfaces, Figure 3b,c.

The capability of LEMS to detect pure pharmaceuticals from highly porous materials was investigated using white, red, and


black polyester fabric. Figure 4a,b shows representative spectra resulting from LEMS analysis of oxycodone from white and red polyester fabric, respectively. White fabric has the highest parent ion signal followed by red. Oxycodone was not detected on black fabric above the limit of detection (S/N > 3). The differences in the signal intensity are likely due to a combination of the porosity, polarizability, and charging of a given surface. The polarity of the polyester backbone presumably forms a strong interaction with oxycodone that is not present on the glass or steel surface. The dye used to color the fabric, for example, the carbon black used in black fabric, will provide additional strong interactions that may serve to further decrease vaporization efficiency. As seen in Figures 3 and 4, the parent molecular ion for oxycodone is detected on glass, metal, wood, and fabric substrates. This suggests that laser vaporization will occur from a variety of surfaces when nonresonant femtosecond vaporization is employed as the release method and that LEMS may form the basis of a universal detection method for organic molecules.

**LEMS of Trace Amounts of Atenolol on Different Surfaces.** To investigate the detection of trace amounts of atenolol on various surfaces, 50 µL of the 1.3 × 10^{-4} M solution was deposited on wood, steel, glass, and fabric with sample coverage area ranging from 12 to 160 mm². The total sample applied to each surface was 1.3 µg. Approximately 430 pmol of atenolol was vaporized during the 50 laser shot acquisition to produce the measured mass spectra. Figure 5 shows representative mass spectra of protonated atenolol on steel and glass. Atenolol was not detected on the wood substrate. The parent ion, [M+H]^+, can be seen at m/z = 267 on both glass and steel surfaces, and the sodium adduct, [M+Na]^+, at m/z = 289 can be seen on the steel surface, Figure 5b. Figure 6a–c shows the detection of atenolol on white, red, and black polyester fabric, respectively. The signal intensity on both wood and fabric are lower compared to the average signal from oxycodone deposited on these materials. Atenolol is more polar than oxycodone and an enhanced interaction with the substrate material may account for the reduced signal, particularly in the case of the polar cellulose matrix composing the wood substrate. In comparison with atenolol, oxycodone will have weaker interactions with the cellulose matrix, facilitating vaporization. The same may be true for the reduced parent ion signal for atenolol on fabric. Protonated atenolol was not detected on the different colored fabrics, but the sodium adduct of atenolol can be seen on all three fabrics.

Table 1 shows the statistical analysis of the mass spectra measured for 20 LEMS experiments on each surface. The amount of sample deposited was 1.3, 1.6, and 1.6 µg for atenolol, loratadine, and oxycodone, respectively. The samples covered 10–90 mm² for glass, 12–60 mm² for metal, 120–170 mm² for both wood and cloth. The average integrated area (IA) is calculated as well as the relative standard deviation (%RSD). The %RSD was determined by dividing the standard deviation (× 100) by the mean. We define the signal-to-noise (S/N) threshold for signal as being greater than three. Claritin tends to have lower %RSD, compared to atenolol and oxycodone. The capability of LEMS to detect pharmaceuticals on glass and steel is high compared to the detection on wood and cloth. Claritin was not detected...
on the polyester fabric while oxycodone was detected on both the white and red fabric.

The relative standard deviation is high for all the surfaces because of the variability in sample preparation from the spot and dry deposition method. The deviation occurs from sample preparation rather than fluctuations in laser intensity, electrospray source, or mass spectrometry ion optics. The sample preparation method results in different amounts of sample ablated from position to position on a sample. Therefore, the %RSD for LEMS experiments are not comparable to the %RSD of traditional analysis techniques where a consistent amount of sample is analyzed for each experiment. However, the samples analyzed in these experiments are more consistent with field conditions.

We note that the steel substrate (0.8 mm in thickness) provides the highest signal intensity for all the molecules investigated. This presumably results from the steel optimally compensating for the distortion of the electric field between the electrospray needle and the inlet capillary. Dielectrics (glass with 1.0 mm thickness) and polymer-based substrates (fabric and wood with 1.65 mm thickness) will have ill-defined field lines due to the buildup of charge in localized areas from both the electrospray and laser vaporization process. These areas of patch charging cannot be adequately discharged due to the nonconducting nature of the substrates. The distortion of the electrospray plume results in a decrease in the observed signal intensity for the laser vaporized molecules. The enhanced signal from steel is unlikely to be due to rapid heating in the metal substrate because any thermal molecules. The enhanced signal from steel is unlikely to be due to rapid heating in the metal substrate because any thermal

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Average IA, N = 20</th>
<th>%RSD</th>
<th>S/N</th>
<th>Average IA, N = 20</th>
<th>%RSD</th>
<th>S/N</th>
<th>Average IA, N = 20</th>
<th>%RSD</th>
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<td>4.2</td>
<td>0.98</td>
<td>48</td>
<td>12</td>
<td>0.19</td>
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<td>39</td>
<td>3.5</td>
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</tr>
<tr>
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<td>34</td>
<td>3.9</td>
<td>0.23</td>
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<tr>
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* Average integrated area (IA) and relative standard deviation (%RSD) are calculated. Average IA for the noise level was 0.062 ± 0.020. ND = not detected.

Neutral Capture Efficiency. To determine the neutral capture efficiency (NCE) of vaporized molecules transferred from the substrate into the electrospray system, the signal arising from LEMS for a known quantity of sample was compared to the calibrated signal arising from direct electrospray mass spectrometry (ESI-MS) measurements. A 50 µL aliquot of 10<sup>-4</sup> M atenolol or oxycodone was deposited on a steel surface to cover an area ranging from 12 to 30 mm<sup>2</sup> for LEMS analysis. Direct ESI-MS measurements were recorded to calibrate the electrospray-mass spectrometer instrument response function to calculate the neutral capture efficiency. The neutral capture efficiency is given by

\[ NCE = \frac{I_{LEMS} \times \text{moles}_{ESI}^{LEMS}}{I_{ESI} \times \text{moles}_{LEMS}^{ESI}} \] (1)

where $I_{LEMS}$ is the integrated signal intensity for the LEMS measurement, moles$_{ESI}$ is the amount of sample consumed for the ESI measurement, $I_{ESI}$ is the integrated signal intensity for the direct ESI-MS measurement, and moles$_{LEMS}$ is the amount of sample consumed for the LEMS measurement. Two assumptions were made in determining the neutral capture efficiency. The first assumption is that all of the captured neutrals have the same probability to be ionized and detected in comparison with sample that has been dissolved in the electrospray solution. This may not be a valid assumption if clusters are vaporized and the clusters do not have sufficient time to dissolve into the droplet solution before the ions enter the vacuum region. The second assumption is that 100% of the adsorbed molecules in the laser interaction region are vaporized. Both of these assumptions serve to make the NCE determined here have a lower limit.

To calculate neutral capture efficiency, we compared the signal intensity of the electrospray spectrum to the LEMS spectrum for the same initial concentration of sample molecule. In this concentration range, the area under the peak is considered proportional to the concentration of the sample. The total moles consumed for the ESI-MS measurement is given by

$$\text{moles}_{ESI} = fCt$$

(2)

where $f$ is the flow rate (2 µL/min), $C$ is the concentration (1.3 $\times$ 10$^{-4}$ M), and $t$ is the analysis time (5 s). In the calibration experiment, 21 pmol of atenolol were consumed for the electrospray mass spectrum. The determination of the sample consumed for LEMS is complicated by the fact that after deposition on the steel surface, the dried sample is not deposited uniformly in the sample area but tends to dry with the majority of molecules contained in a ring. Therefore, the assumption of uniform sample per unit area is not valid. Rather, we determined the amount of sample dried in, and vaporized from, the rings. To determine the amount of sample dried in the ring, a magnified picture of the sample slide was taken using a low power microscope. The picture of the sample was then imported into an image software program (ImageJ) to integrate both the number of black pixels (representing sample) and white pixels (representing the stainless steel substrate) in the area where sample was deposited (see Figure S2 in Supporting Information). The amount of sample found only in the ring can then be determined by restricting the integration to the area containing sample, the black pixels. This provides the area of the sample ring. After the sample was vaporized using LEMS (after 5 s of raster scanning), a second image of the sample was recorded. The amount of ablated sample is determined by comparing the amount of sample before and after vaporization. For the LEMS measurement of atenolol, 0.5–1 nmol (1.3 $\times$ 10$^{-4}$ M solution) was vaporized from the steel surface. Given the ratio of the signal intensity for ESI-MS and LEMS measurements, we conclude that not all of the molecules vaporized with femtosecond pulses are captured in the electrospray plume. Neutral capture efficiencies for oxycodone and atenolol on steel are 2.4 ± 1.5% and 0.25 ± 0.18%, respectively. The neutral capture efficiency of 0.02% has been reported for nanosecond MALDESI of 10$^{-4}$ M ubiquitin using a matrix on stainless steel substrates. This calculation was based on the amount of material spotted, the size of the spot, the laser spot size, the number of laser shots, and the absolute abundance of ubiquitin relative to its corresponding nano-ESI spectra. We have demonstrated neutral capture efficiencies as high as 2.4% using femtosecond pulses without applying an external matrix. This suggests that intact, neutral molecules are efficiently vaporized using femtosecond lasers in comparison with nanosecond lasers since the NCE ranges from 12 to 100 times higher.

CONCLUSIONS

We have demonstrated that nonresonant, femtosecond laser vaporization is capable of analyzing intact pharmaceutical formulations without sample preparation and in the presence of binders and fillers. We demonstrated that LEMS is a new
release technology for detecting pharmaceutical molecules that may be found on glass, wood, steel, and fabric. Detection of pharmaceuticals from a variety of surfaces suggests that the method can be extended to a wide variety of controlled substances. Because there is essentially no sample preparation, the method has extremely short analysis time (s) and the ability for detection of trace quantities from native samples. The neutral capture efficiency in LEMS is 12 to 100 times higher in comparison with nanosecond MALDESI. We have demonstrated that LEMS is capable of producing a mass spectral image of a sample under ambient condition with a spatial resolution of 250 μm.

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