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# Thin-Layer Chromatography/Laser-Induced Acoustic Desorption/Electrospray Ionization Mass Spectrometry

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The combination of laser-induced acoustic desorption and electrospray ionization mass spectrometry (LIAD/ESI/MS) can be used to rapidly characterize chemical compounds separated on a thin layer chromatography (TLC) plate. We performed LIAD analysis by irradiating the rear side of an aluminum-based TLC plate with a pulsed infrared (IR) laser. To efficiently generate and transfer acoustic and shock waves to ablate the analyte-containing TLC gels, a glass slide was attached to the rear of the TLC plate and the gap between the glass slide and the TLC plate was filled with a viscous solution (glycerol). Although the diameter of the laser spot created on the rear of the TLC plate was approximately 0.35 mm, the ablated areas on the front sides of the silica gel bed and the C<sub>18</sub> reverse-phase gel bed had diameters of approximately 1.3 and 3 mm, respectively. The ablated analyte molecules were ionized in an ESI plume and then detected by an ion trap mass analyzer. This TLC/LIAD/ESI/MS approach allowed the components in mixtures of dye standards, drug standards, and rosemary essential oil to be separated and rapidly characterized.

Thin-layer chromatography (TLC), in which the mobile phase is driven by capillary action rather than a gas stream or liquid pumping system, is a simple and economical technique for separating chemical mixtures. The separation process relies on noncovalent interactions between the analyte molecules with the solid adsorbers coated on the TLC plate during development.<sup>1–5</sup> Optical measurement and visualization are typical methods used to detect separated compounds on a TLC plate; the specificity of these detection methods, however, remains low. The application of mass spectrometry (MS), with its high sensitivity and utility in

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structural analysis, to the characterization of chemical compounds on TLC plates has been of interest for several years.<sup>6–8</sup> Conventionally, the compound separated on a TLC plate is scraped from the surface, extracted, and heated inside the ion source under vacuum and ionized through electron impact (EI) or chemical ionization (CI).<sup>9–11</sup> Because the analyte is evaporated through heating, these techniques are limited to samples that are thermally stable and volatile. Desorption ionization (DI) techniques, such as fast atom bombardment (FAB), secondary ion mass spectrometry (SIMS), laser desorption (LD), and matrix-assisted desorption ionization (MALDI), have been used to characterize nonvolatile or thermally labile compounds directly on TLC plates.<sup>12–17</sup> Nevertheless, several problems are still encountered when using these approaches: (1) time and labor consumption, due to the requirement of a high vacuum for the ion source; (2) poor sensitivity for the detection of volatile or semivolatile compounds; (3) interference from small MALDI matrix ions; (4) relatively poor reproducibility in quantitative analyses; and (5) lateral diffusion of analytes after depositing the MALDI matrix solution on the TLC plate.

To avoid interference from the matrix chemicals, related electrospray techniques (without using a matrix) have been reported for the coupling of TLC and MS. Van Berkel and co-workers introduced a TLC/ESI/MS system that exploited a surface sampling probe-to-TLC plate liquid microjunction and a self-aspirating electrospray emitter for the direct detection of small organic compounds on TLC plates.<sup>18–20</sup> Luftmann developed a

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plunger-based extraction device for TLC/ESI/MS.<sup>21</sup> We have employed a direct electrospray probe to generate analyte ions directly from the end of a TLC plate.<sup>22</sup> Nevertheless, such TLC/ESI interfaces can suffer from clogging of the capillary used to transfer the analyte-containing liquid to the electrospray source. These interfaces are also complicated to construct and operate.

Ambient MS techniques have been developed recently to create ions in their native environments without the need for additional preparation processes.<sup>23,24</sup> The ionization sources included in this category are desorption electrospray ionization (DESI), direct analysis in real time (DART), atmospheric pressure solids analysis probe (ASAP), electrospray-assisted laser desorption ionization (ELDI), and laser-induced acoustic desorption/electrospray ionization (LIAD/ESI).<sup>25–32</sup> Because TLC is also performed under ambient conditions, it is much easier to couple TLC to ambient MS than to vacuum-based ionization sources. Among the ambient ionization sources, desorption-based sources (e.g., ELDI, DESI) have high spatial resolution for sampling, making them particularly suitable for characterizing compounds within a small TLC spot. Van Berkel et al. used a DESI apparatus to successfully couple TLC with MS.<sup>33</sup> We have employed ELDI to directly characterize organic compounds separated on a TLC plate.<sup>34</sup>

Laser-induced acoustic desorption (LIAD) is a technique developed for desorbing and ionizing organic and biological materials from solid substrates under vacuum.<sup>35–38</sup> After irradiation of a pulsed laser beam to the rear of a thin metal foil, the resulting ablation creates large-amplitude acoustic and shock waves that propagate through the foil. It has been suggested that

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molecules predeposited on the other side of the foil would be detached through the actions of the acoustic and shock waves, with the rapid expansion of the shock wave ensuring that the molecules do not break apart.<sup>35–38</sup> Although ionization of the analytes does occur during LIAD, the ion yields remain low. The ion yields can be improved, however, when postionizing the neutral molecules desorbed by LIAD through EI, CI, or photo-ionization under vacuum.<sup>39–41</sup> The desorption/ionization efficiency of large molecules (e.g., proteins) can be improved by mixing the protein molecules with a MALDI matrix prior to performing LIAD processes.<sup>37</sup> Even under these conditions, the mass range of the analyte ions suitable for LIAD MS analysis is limited to less than *m/z* 20 000.<sup>37</sup>

We have developed an ambient technique that combines LIAD with ESI MS.<sup>32</sup> The ionization mechanisms in the LIAD/ESI source are similar to those used for fused-droplet electrospray ionization (FD-ESI)<sup>30,42–44</sup> and so-called extractive electrospray ionization (EESI),<sup>45</sup> i.e., the neutral molecules or fine droplets desorbed by the LIAD processes are ionized through fusion with charged solvent droplets, followed by ESI or ion–molecule reactions with charged solvent species in the ESI plume. Ambient LIAD/ESI provides several benefits: (1) sample switching is rapid, (2) both liquids and solids can be analyzed, and (3) the detected mass range is large (*m/z* 66 000 for albumin ion).<sup>32</sup>

Although the capability of LIAD to desorb chemical compounds adsorbed onto TLC gel particles has not been reported, the “shaking” effect induced by LIAD processes has been used to efficiently desorb analyte molecules from several other solid surfaces.<sup>32,35–41</sup> Therefore, we suspected that molecules adsorbed onto TLC gel particles would also be desorbed through LIAD. One of the potential advantages of coupling LIAD/ESI/MS with TLC would be that photosensitive compounds would remain undamaged during analysis, because the laser light is irradiated onto the rear of the TLC plate, rather than its front. Comparing to ELDI, the working distance between the laser and the TLC plate in LIAD/ESI is greatly reduced. The construction of an interface for TLC/LIAD/ESI should then be easier and simpler than that for TLC/ELDI. In this study, we constructed a LIAD/ESI source and employed it to characterize analytes separated on TLC plates, which featured either reversed-phase C<sub>18</sub> particles or normal-phase silica gel coating on an aluminum-based foil. The samples used for the ambient LIAD/ESI/MS detection of analytes separated on TLC plates included a mixture of dye standards, drug standards, and rosemary essential oil.

## EXPERIMENTAL SECTION

**Materials.** Dye standards (FD&C Green No. 3, FD&C Red No. 3, and eriochromcyanin R) were purchased from Sigma-Aldrich (St Louis, MO) and Merck (Darmstadt, Germany); drug

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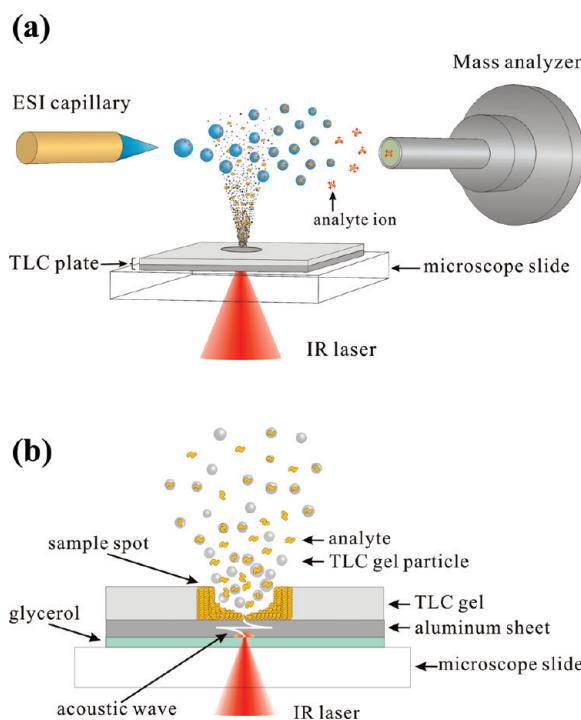
standards [3,4-methylenedioxy-N-methamphetamine (MDMA), lysergic acid diethylamide (LSD), flunitrazepam (FM2)] were purchased from Cerilliant (Round Rock, TX); rosemary essential oil was purchased from a local supermarket. Methanol, acetone, toluene, and ethyl acetate (HPLC or GC grade) were purchased from Merck; acetic acid and glycerol were purchased from J. T. Baker (reagent grade, Phillipsburg, NJ). Distilled deionized water was used for sample preparation or pretreatment (produced by Milli-Q plus, Millipore; Molsheim, France). Aluminum TLC plates coated with reversed-phase C<sub>18</sub> (RP-18 F<sub>254</sub>S) and normal-phase silica (silica 60 F<sub>254</sub>) gel particles were purchased from Merck. The dimensions of the TLC plate were 6 cm × 5 cm (WL), the aluminum sheet was 100 μm thick, and the C<sub>18</sub> or silica gel layer was 200 μm thick. Microscopic glass slides (2.5 cm × 7.5 cm; thickness 1–1.2 mm) were purchased from FEA.

**TLC Separation.** A methanol solution containing three dye standards was prepared. The sample solution (1 μL) was spotted on a reversed-phase C<sub>18</sub> TLC plate. The concentrations of the dye standards in the sample solution were 10<sup>-3</sup> M for the FD&C Red No. 3, 10<sup>-3</sup> M for the FD&C Green No. 3, and 2 × 10<sup>-3</sup> M for eriochromcyanin R. The TLC plate was developed in 65% acetone solution containing 1.5% formic acid. The plate was then air-dried for 1 h prior to LIAD/ESI/MS analysis.

A methanol solution containing a mixture of three drug standards—MDMA, LSD, and FM2—was spotted in 1-μL aliquots at the center of a normal-phase silica gel TLC plate. The concentration of each drug in the sample solution was 33.3 μg/mL. A mixture of chloroform and methanol (9:1, v/v) was used as the mobile phase for developing the drug standards on the TLC plate.

A normal-phase silica gel TLC plate was used to separate the components in rosemary essential oil (1 μL), which was spotted on the TLC plates without pretreatment. The TLC plate was then developed using a mixture of toluene and ethyl acetate (9:1, v/v). Photographs of the developed TLC plates were taken using digital cameras (EOS 350D, Canon or G20, X-Loupe) under white light or UV illumination (UVGL-58, UVP).

**TLC/LIAD/ESI/MS System.** After development and air-drying, the TLC plate was set on an XYZ stage for LIAD/ESI/MS analysis. In one case, a thin film of glycerol (approximately 10 μL) was applied evenly to the rear of a cut TLC plate [1.5 × 5 cm (WL)] and the plate then tapped tightly on a microscope glass slide; the TLC plate was stabilized together with the glass slide on a stage and then positioned in front of an ion trap mass spectrometer. The sample stage was moved by a robotic platform at a rate of 0.01–0.02 cm/s. Figure 1 provides a schematic representation of the ambient TLC/LIAD/ESI system. After being focused by a single lens, a pulsed Q-switched Nd:YAG laser beam (LS-2130, LOTIS TII, Belarus, Russia) transmitted through the microscope glass slide and irradiated the rear side of the TLC plate (i.e., the aluminum sheet). The laser was operated at a wavelength of 1064 nm, a frequency of 1 or 2 Hz (controlled by a sweep function generator), a pulsed energy of 1.1–28 mJ (measured offline using a laser power and energy meter, SOLO 2, Gentec-EO), a pulse duration of 9 ns, and a laser spot diameter of 350 μm. The power densities (PD) were calculated with PD = (laser energy)/(pulse duration × spot area).



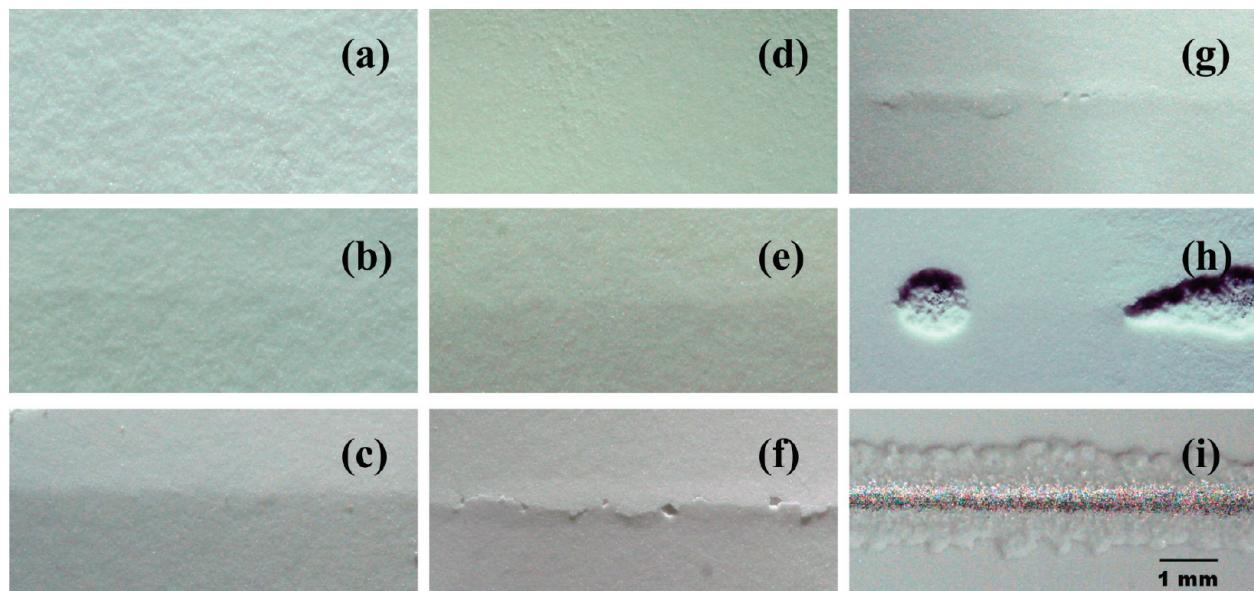
**Figure 1.** (a) Schematic illustration of the LIAD ESI approach to ablate and ionize organic compounds separated on a TLC plate and (b) a close view of the ablation process.

The configuration of the ambient LIAD/ESI source was similar to those of ELDI, pyrolysis/ESI, and FD-ESI sources.<sup>30,42–44,46</sup> The capillary electrospray was aligned parallel to and 2–3 mm above the sample spot surface on the TLC plate. The ESI plume was directed toward the ion sampling tube on an ion trap mass analyzer (esquire 3000+, Bruker Daltonics). The electrospray solutions were methanol/water (1:1, v/v) solution with 0.1% acetic acid (for positive ion detection) and 100% methanol (for negative ion detection). The flow rate of the ESI solution in the capillary was 2.5 μL/min. A nebulizing gas, commonly used in conventional ESI sources, was not used during LIAD/ESI analysis. The resulting analyte ions were sampled into the ion trap mass spectrometer. The voltage applied at the ion sampling tube was −500 V for positive ion detection and +500 V for negative ion detection. The voltage applied at the electrospray needle was +1.7 kV for positive ion detection and −1.5 kV for negative ion detection.

## RESULTS AND DISCUSSION

In this study, the laser energy in a LIAD/ESI source was used mainly to ablate the analyte-absorbed gel particles from the TLC plate. This setup is different from that described in our previous LIAD/ESI/MS study,<sup>32</sup> where the laser energy was used to desorb the analyte molecules from the surface of the liquid or solid deposited on a metal foil. Here, we examined the ablation of the silica gel particles by irradiating the rear side of the plate (i.e., aluminum sheet) with a pulsed IR laser beam. We found that even when the laser energy was increased from 1.1 to 28 mJ (laser power density ranges from  $1 \times 10^8$  to  $2.5 \times 10^9$  W/cm<sup>2</sup>), no silica gel particles were removed from the TLC plate. The photo-

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**Figure 2.** Photographs of silica gel surface on the TLC plates after performing the LIAD process. A pulsed IR laser irradiated the rear of three TLC plates ( $100\ \mu\text{m}$  aluminum foil): (a–c) without anything attached, (d–f) with a microscope glass slide attached, and (g–i) with a microscope glass slide attached and the gap between the glass slide and the aluminum foil filled with glycerol. The laser power densities were  $3.9 \times 10^8\ \text{W}/\text{cm}^2$  (a, d, g),  $7.9 \times 10^8\ \text{W}/\text{cm}^2$  (b, e, h), and  $2.1 \times 10^9\ \text{W}/\text{cm}^2$  (c, f, i).

graphs in Figure 2a–c display the surface of the silica gel bed after LIAD analysis; the individual laser power densities were  $3.9 \times 10^8\ \text{W}/\text{cm}^2$  (Figure 2a),  $7.9 \times 10^8\ \text{W}/\text{cm}^2$  (Figure 2b), and  $2.1 \times 10^9\ (\text{Figure } 2\text{c})\ \text{W}/\text{cm}^2$ , referring to the irradiation energy of 4.3, 8.7, and 23 mJ. The central line of the TLC plate was slightly protruded (Figure 2c), but the surface remained nearly undamaged. No analyte ions were detected under such high laser energies when using the TLC/LIAD/ESI/MS system (data not shown).

When the surface of the silica gel bed was irradiated directly with the IR laser beam (as in the ELDI process), the gel bed was totally ablated away when the laser energy reached 23 mJ. This observation suggests that, in the LIAD process, the laser energy transferred from the rear side of the TLC plate to the silica gel bed by the acoustic wave is far too low to ablate the gel particles. This low-efficiency energy transfer may be due to the acoustic wave being offset by the vibration of the TLC plate during laser irradiation.

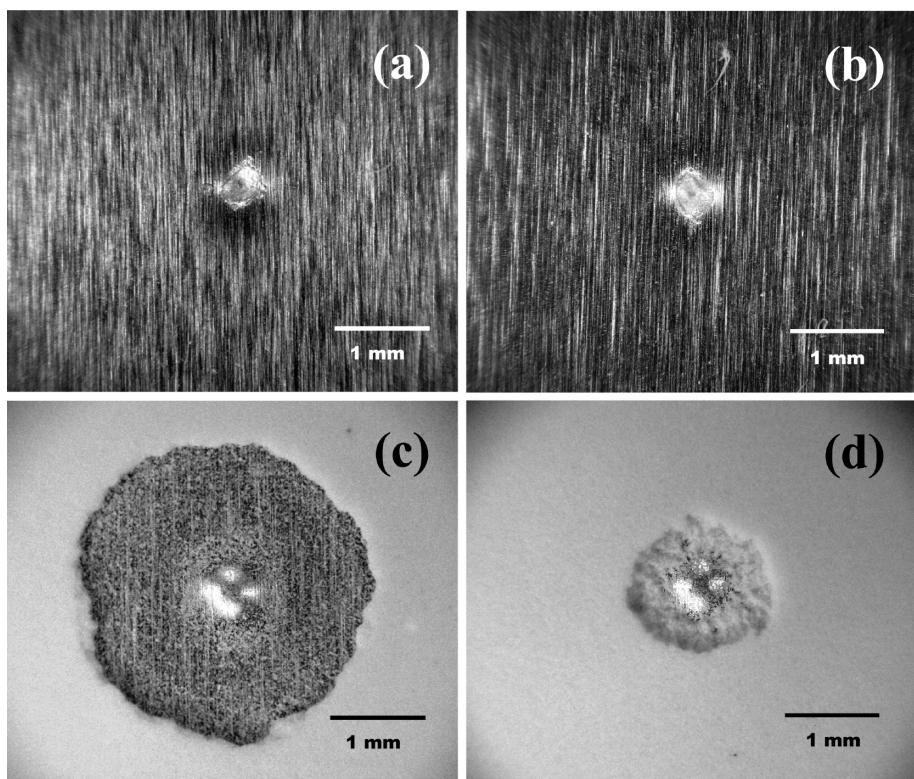
To enhance the energy transfer efficiency, by preventing the vibration on the TLC plate, we tightly taped the TLC plate onto a glass microscope slide (Figure 2d–f). Again, however, similar to the result displayed in Figure 2c, the surface of the TLC plate remained nearly undamaged when the laser power density was less than  $2.1 \times 10^9\ \text{W}/\text{cm}^2$  (Figure 2d,e). Nevertheless, when the laser power density was greater than  $2.1 \times 10^9\ \text{W}/\text{cm}^2$ , a hump was created on the silica gel bed surface (Figure 2f). Although we observed very few gel particles intermittently detached from the TLC plate during the LIAD processes, no signals for analyte ions were detected by the TLC/LIAD/ESI/MS system.

To further enhance the energy transfer efficiency, we filled the space between the glass slide and the TLC plate with glycerol, simply by applying a thin film of glycerol to the glass slide prior to attaching it to the TLC plate (Figure 2g–i). Similar to the results displayed in Figure 2f, a hump was created on the silica gel bed surface when the power density was  $3.9 \times 10^8\ \text{W}/\text{cm}^2$  (Figure

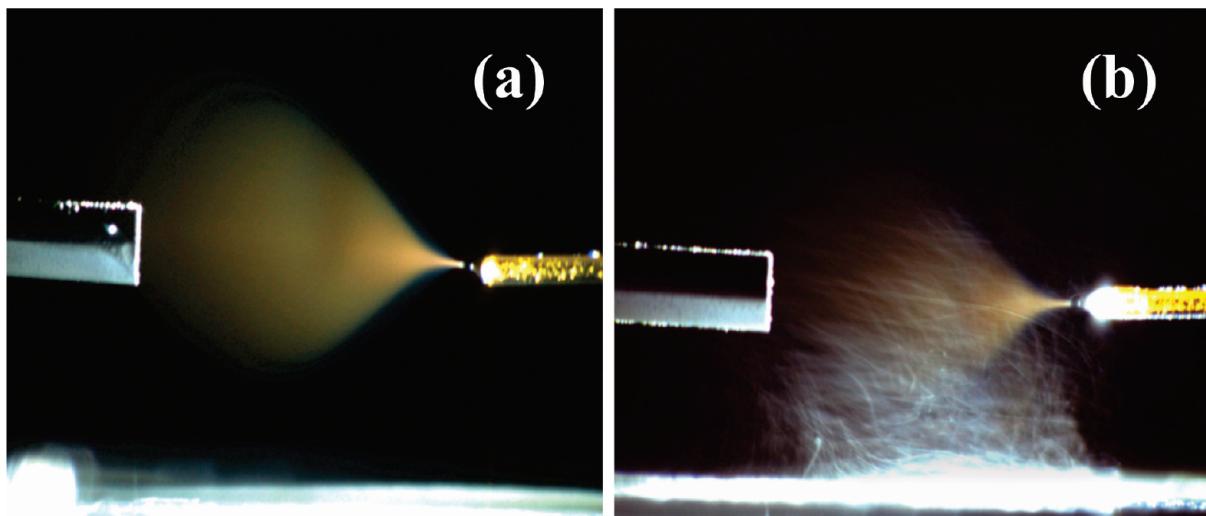
2g). As the laser power density was increased to  $7.9 \times 10^8\ \text{W}/\text{cm}^2$ , the silica gel on the TLC plate was intermittent ablated by the LIAD process (see the two big craters on the TLC plate in Figure 2h). The silica gel on the TLC plate was efficiently ablated by the LIAD process when the power density was greater than  $2.1 \times 10^9\ \text{W}/\text{cm}^2$ . Figure 2i reveals that the surface of the silica gel was broken and the gel particles were ablated from the plate. We conclude that, with the help of the glass slide and glycerol, the energy generated by the LIAD system was efficiently transferred from the rear of the aluminum sheet to the gel bed on the front of the TLC plate. Although the IR laser energy was presumably partially absorbed by glycerol, strong acoustic and shock waves were still generated to efficiently ablate the gel particles. In the following experiments, the TLC plate was always tightly attached to a microscopic glass slide with glycerol and the laser energy for LIAD processes was set at 23 mJ. No glycerol ions were detected on the LIAD/ESI mass spectrum.

To study the size of the ablated gel bed during LIAD analysis, the rear of the TLC plate was irradiated with a single IR laser shot. Figure 3 displays photographs of the rear and front sides of the TLC plates after LIAD analysis. We examined both  $\text{C}_{18}$  (reverse phase) and regular silica gel (normal phase) as TLC coatings. The diameter of the laser spot on the aluminum sheet was best focused at approximately 0.35 mm. We obtained circularly ablated areas having diameters of approximately 3 and 1.3 mm on the reverse- and normal-phase silica gel beds, respectively, suggesting that the  $\text{C}_{18}$  gel bed was easier to remove than the regular silica gel bed.

The photographs in Figure 4 display the ablation of gel particles from the silica TLC plate during the LIAD/ESI processes. Figure 4a presents the electrospray plume and the surface of the TLC bed in the absence of laser irradiation. Figure 4b reveals that numerous particles were generated when the rear of the TLC plate was irradiated with the pulsed IR laser. The bright trajectories represent the large particles leaving the TLC bed. Careful



**Figure 3.** Photographs of TLC plates after irradiation with a pulsed IR laser: (a) rear side of a TLC plate (aluminum foil) coated with  $C_{18}$  gel, (b) rear side of a TLC plate (aluminum foil) coated with silica gel, (c) front side of a TLC plate coated with  $C_{18}$  gel, and (d) front side of a TLC plate coated with silica gel.

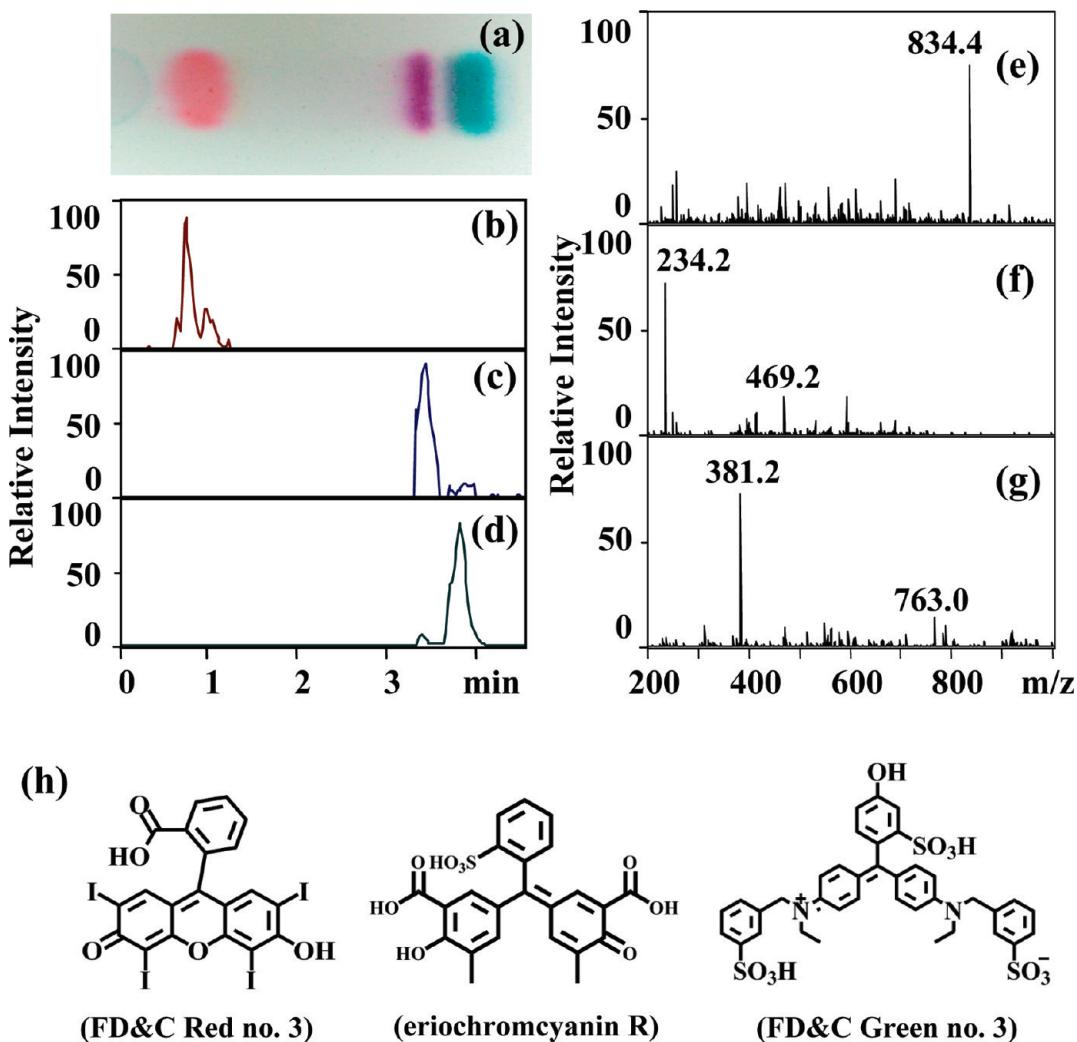


**Figure 4.** Photographs of the surface of a silica TLC plate in the LIAD/ESI source when (a) the laser for LIAD analysis was switched off (the ESI plume located 2 mm above the TLC plate is evident) and (b) the detached silica gel particles join the ESI plume when the pulsed IR laser for LIAD was switched on.

examination of the surface of the plate with the naked eye revealed numerous, but faint, fine particles generated at the surface of the silica bed. When these particles entered the ESI plume, which was positioned approximately 2 mm above the TLC plate, we suspect that the analytes absorbed onto the particles dissolved in the charged solvent droplets and were then postioned by the ESI processes that proceeded from the analyte-containing charged droplets. It is also possible that the analyte molecules were desorbed directly from the TLC gel by the action of the acoustic

wave and that these molecules then interacted with the charged species in the ESI plume to produce analyte ions (ion–molecule reactions).

To directly characterize small analyte molecules separated on the TLC plate, we employed dye standards, drug standards, and rosemary essential oil as the samples to test the LIAD/ESI/MS method. The highest  $m/z$  value of the standard is 834.4 (FD&C Red No. 3). We first used a reversed-phase  $C_{18}$  gel-coated TLC plate to separate a mixture of dye standards (FD&C Red No.

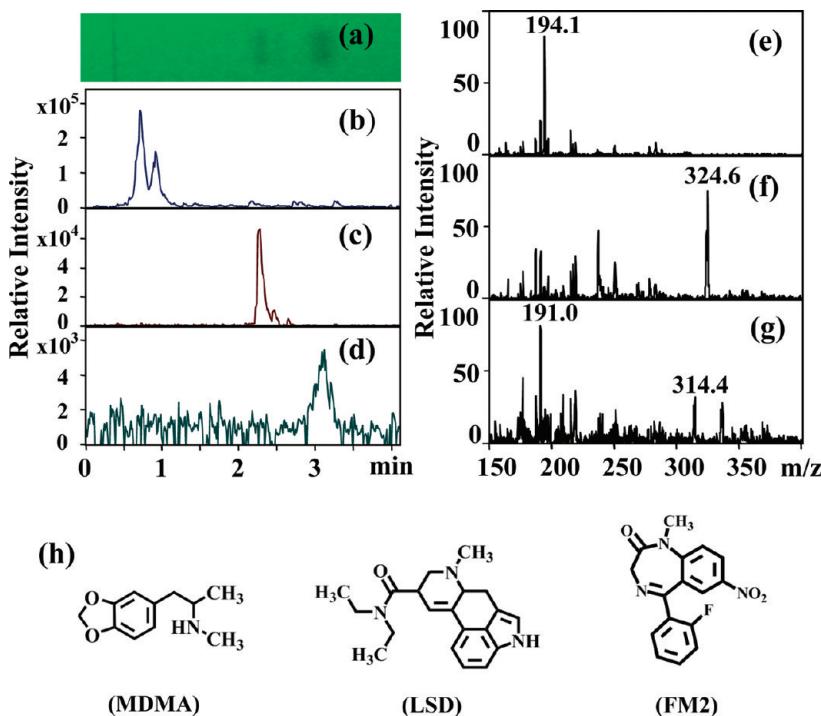


**Figure 5.** (a) Photograph of three separated dye standards on a C<sub>18</sub>-coated TLC plate. Extracted ion chromatograms of (b) FD&C Red No. 3 ( $[M - H]^-$ ,  $m/z$  834.4), (c) eriochromcyanin R ( $[M - 2H]^{2-}$ ,  $m/z$  234.2), and (d) FD&C Green No. 3 ( $[M - 3H]^{3-}$ ,  $m/z$  381.2). Negative-ion LIAD/ESI mass spectra of (e) FD&C Red No. 3, (f) eriochromcyanin R, and (g) FD&C Green No. 3. (h) Structures of FD&C Red No. 3, eriochromcyanin R, and FD&C Green No. 3.

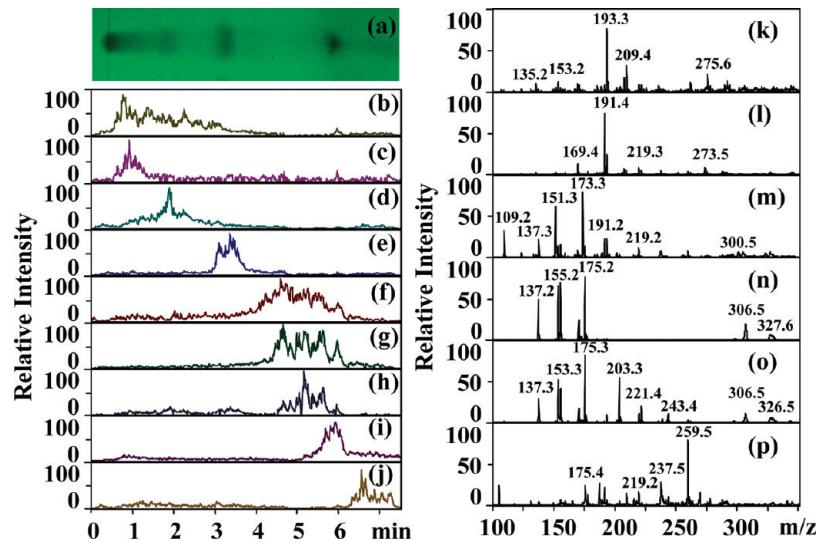
3, FD&C Green No. 3, and eriochromcyanin R). Figure 5a presents a photograph of the developed sample lane on the TLC plate. After development, the three sample spots were completely resolved, with values of  $R_f$  [(the migration distance of analyte)/(the migration distance of solvent front)] of 0.23 for the FD&C Red No. 3, 0.76 for eriochromcyanin R, and 0.88 for the FD&C Green No. 3. The TLC plate/glycerol/glass slide assembly was then positioned in front of the MS inlet of an ion trap mass analyzer and the rear side of the central unit was linearly scanned by the pulsed IR laser. The ions generated by the LIAD/ESI process were then detected by an ion trap mass spectrometer operated in negative ion mode. Figure 5b–d presents the extracted ion chromatograms of the FD&C Red No. 3 ( $m/z$  834), eriochromcyanin R ( $m/z$  234), and the FD&C Green No. 3 ( $m/z$  381). Figure 5e–g displays the LIAD/ESI mass spectra recorded at each spot of the dye standard mixture. The dye standards were detected either as singly charged ( $m/z$  834.5 for FD&C Red No. 3,  $m/z$  469.2 for eriochromcyanin R,  $m/z$  763 for FD&C Green No. 3) or doubly charged ( $m/z$  234.2 for eriochromcyanin R,  $m/z$  381.2 for FD&C Green No. 3) ions.

The results in Figure 5 clearly indicate that combining TLC/glycerol/glass slide assemblies with LIAD/ESI/MS allowed the detection of analytes separated on a reverse-phase TLC plate. Because LIAD/ESI has been used to generate analyte ions from the surfaces of other solids,<sup>32</sup> we suspected that the analyte molecules adsorbed on the TLC gel particles might also have been ionized through similar pathways. Therefore, presumably, when the laser-ablated gel particles entered the ESI plume, the analyte molecules rapidly dissolved in the charged solvent droplets and the ESI process then proceeded from the analyte-containing charged droplets to generate analyte ions and ESI-like mass spectra. It is also possible that some of the analyte molecules had desorbed directly from the TLC gel through the action of the acoustic wave; in this case, the desorbed analyte molecules interacted with the charged species in the ESI plume (including protons, hydronium ions, and their cluster ions, solvent ions, and charged droplets) to produce analyte ions.

We selected a normal-phase silica gel TLC plate to separate a mixture of drug standards (MDMA, LSD, and FM2). After separation, the developed TLC plate was illuminated with an UV



**Figure 6.** (a) Photograph of three separated drug standards on a silica gel-coated TLC plate. Extracted ion chromatograms of (b) MDMA, ( $m/z$  194,  $MH^+$ ), (c) LSD ( $m/z$  324,  $MH^+$ ), and (d) FM2 ( $m/z$  314,  $MH^+$ ). Positive-ion LIAD/ESI mass spectra of (e) MDMA, (f) LSD, and (g) FM2. (h) Structures of MDMA, LSD, and FM2.



**Figure 7.** (a) Photograph of the separated components of rosemary essential oil on a silica gel-coated TLC plate. Extracted ion chromatograms of the ions of at  $m/z$  (b) 193, (c) 332, (d) 191, (e) 173, (f) 155, (g) 175, (h) 327, (i) 203, and (j) 260. Positive-ion LIAD/ESI mass spectra recorded at  $R_f$  values of (k) 0.04, (l) 0.19, (m) 0.39, (n) 0.62, (o) 0.76, and (p) 0.92.

lamp (wavelength 254 nm) to visualize the sample spots. Figure 6a displays a photograph of the developed TLC plate prior to LIAD/ESI/MS analysis, revealing the successful separation of the three drug compounds ( $R_f$  of MDMA, LSD, and FM2: 0.04, 0.36, and 0.51, respectively). Figure 6b–d presents the extracted ion chromatograms of MDMA ( $MH^+$ ,  $m/z$  194.1), LSD ( $MH^+$ ,  $m/z$  324.6), and FM2 ( $MH^+$ ,  $m/z$  314.6); Figure 6e–g displays the positive-ion LIAD/ESI mass spectra of the respective analytes. It seems that the proton affinity of FM2 and LSD molecule is lower than that of MDMA, which leads to a lower S/N ratio on their mass spectra (Figure 6f,g). A similar situation also occurred for FD&C Red No. 3 (Figure 5e).

We chose rosemary essential oil, a mixture of chemicals extracted from various plants, to test the applicability of using TLC/LIAD/ESI/MS for the analysis of a sample having a complex composition. After developing the oil on a normal-phase silica gel TLC plate, we visualized the plate under a UV lamp (254 nm). Figure 7a displays the photograph of the developed TLC lane prior to LIAD/ESI/MS analysis of the plate. We detected more than 20 analyte spots on the plate. Figure 7b–j displays the extracted ion chromatograms of nine representative chemical compounds detected on the TLC plate ( $m/z$  193, 332, 191, 173, 155, 175, 327, 203, and 260) at various  $R_f$  values. Figure 7k–p presents positive-ion TLC/LIAD/ESI mass spectra recorded at values of  $R_f$  of 0.04,

0.19, 0.39, 0.62, 0.76, and 0.92. Because the composition of the sample is very complicated, several ion peaks were detected on each spectrum. The identification of these ions using this TLC/LIAD/ESI/MS approach is difficult because of the lack of suitable databanks and libraries of rosemary essential oil. Nevertheless, upon comparison with the extracts of sweet marjoram, turkish oregano, and dittany, Møller et al. have suggested that the signals at  $m/z$  137 and 153 (Figure 7m–o) belong to unique ions (i.e.,  $\alpha$ -pinene and camphor) from rosemary extracts.<sup>47</sup> A compound providing a signal at  $m/z$  151 (Figure 7m) was also reported in a previous study of rosemary extracts (i.e., thymol).<sup>48,49</sup> Therefore, we tentatively assign the signals at  $m/z$  137, 151, and 153 that we detected in the rosemary essential oil to belong to the ions of  $\alpha$ -pinene, thymol, and camphor.<sup>47–49</sup>

## CONCLUSION

We used ambient LIAD/ESI/MS to directly characterize chemical compounds separated on TLC plates coated with reverse-phase C<sub>18</sub> or normal-phase silica gel. With the assistance of a microscopic glass slide and glycerol, the acoustic wave was

efficiently transferred from the rear of the TLC plate to the C<sub>18</sub> or silica gel bed. The analyte molecules adsorbed on the gel particles were subsequently ionized in the ESI plume to form singly or doubly charged analyte ions. The coupling of TLC with LIAD/ESI/MS has several advantageous characteristics: (1) relative to other vacuum-based ionization methods, the construction of an interface to couple TLC with LIAD is easy because both techniques are performed at atmospheric pressure; (2) the size of the TLC plate is not limited by the dimensions of the ion source in the vacuum chamber; (3) both volatile and semivolatile compounds are detectable for analyses performed under ambient conditions; (4) sample switching is rapid. In addition, because the use of organic and inorganic matrices is unnecessary for LIAD/ESI/MS, sample preparation is simple and lateral diffusion of the developed lane on the TLC plate surface is avoided.

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