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Technical Notes

Using Electrospray-Assisted Laser Desorption/Ionization Mass Spectrometry To Characterize Organic Compounds Separated on Thin-Layer Chromatography Plates

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Electrospray-assisted laser desorption/ionization (ELDI), an ionization method that combines laser desorption and electrospray ionization (ESI), can be used under ambient conditions to characterize organic compounds (including FD&C dyes, amines, extracts of a drug tablet) separated in the central track on a thin-layer chromatography (TLC) plate coated with either reversed-phase C_{18} particles or normal-phase silica gel. After drying, the TLC plate was placed on an acrylic sample holder set in front of the sampling skimmer of an ion trap mass analyzer. The chemicals at the center of the TLC plate were analyzed by pushing the sample holder into the path of a laser beam with a syringe pump. The molecules in the sample spot were desorbed by continuously irradiating the surface of the TLC plate with a pulsed nitrogen laser. Then, the desorbed sample molecules entered an ESI plume where they were ionized through the reactions with the charged species (including protons, hydronium ions and their cluster ions, solvent ions, and charged droplets) generated by electrospraying a methanol/water solution. MS/MS analyses were also performed to further characterize the analytes. The detection limit of TLC/ELDI/MS is $\sim 10^{-6}$ M. This was evaluated by using FD&C red dye as the standard. A linear relationship was found for the calibration curve with the concentration of FD&C red dye ranged from 10^{-3} to 10^{-6} M.

Thin-layer chromatography (TLC) is a rapid, simple, and economical separation technique for most organic, inorganic, and biochemical mixtures.[1−5] Although largely a mature technology, improving the techniques for direct coupling of TLC to detective instruments for the rapid detection and identification of trace sample compounds continues to be an active area of investigation.[6,7] In this regard, the direct coupling of TLC and mass spectrometry (MS) is of particular interest because of the latter's high sensitivity, rapid analysis, and ability to aid structural characterization.[8−12] Several previous reports have described the detection of chemical compounds separated by TLC using MS in conjunction with inlet/ion sources inherently amenable to the surface analysis challenges presented by TLC. For example, molecules separated on TLC plates have been scratched and heated inside the ion source under vacuum and then ionized through electron impact or chemical ionization.[13,14] Because these conventional ionization methods require heating of the analyte, they are suitable only for the analyses of volatile and thermally stable compounds. In contrast, desorption/ionization (DI) techniques, such as fast atom bombardment, plasma desorption, laser desorption, and matrix-assisted laser desorption/ionization (MALDI),[15−20] appear to be better methods for directly characterizing nonvolatile

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(1) York, H.; Funk, W.; Fischer, W.; Wimmer, H. *Thin-Layer Chromatography*; VCH: New York, 1990.
(2) Geiss, F. *Fundamentals of Thin Layer Chromatography (Planar Chromatography)*; Huethig: Heidelberg, 1987.
(3) Sherma, J. *Anal. Chem.* **2000**, *72*, 9R-25R.
(4) Fried, B.; Sherma, J. *Thin-Layer Chromatography*, 4th ed.; Marcel Dekker: New York, 1999.
(5) Sherma, J. *Anal. Chem.* **2002**, *74*, 2653−2662.
(6) Poole, C. *J. Chromatogr., A* **2003**, *1000*, 963−984.
(7) Poole, C. *J. Chromatogr., A* **2000**, *856*, 399−427.
(8) Busch, K. L. *J. Chromatogr., A* **1995**, *692*, 275−290.
(9) Wilson, I. D.; Morden, W. *J. Planar Chromatogr.* **1996**, *9*, 84−91.
(10) Wilson, I. D. *J. Chromatogr., A* **1999**, *856*, 429−442.
(11) Gusev, A. I. *Fresenius' J. Anal. Chem.* **2000**, *366*, 691−700.
(12) Wilson, I. D. *J. Chromatogr., A* **1999**, *856*, 429−442.
(13) Busch, K. L. In *Handbook of Thin-Layer Chromatography*; Sherma, J., Fried, B., Eds.; Marcel Dekker: New York, 1990.
(14) Kaiser, R. *Chem. Br.* **1969**, *5*, 54−61.
(15) Didonato, G. C.; Busch, K. L. *Anal. Chem.* **1986**, *58*, 3231−3232.
(16) Monaghan, J. J.; Morden, W. E.; Johnson, T.; Wilson, I. D.; Martin, P. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 608−615.
(17) Danigel, H.; Schmidt, L.; Jungclas, H.; Pfluger, K. H. *Biomed. Mass Spectrom.* **1985**, *12*, 542−545.
(18) Kubis, A. J.; Somayajula, K. V.; Sharkey, A. G.; Hercules, D. M. *Anal. Chem.* **1989**, *61*, 2516−2523.
(19) Mehl, J. T.; Hercules, D. M. *Anal. Chem.* **2000**, *72*, 68−73.

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and thermally labile chemical or biochemical compounds separated by TLC.[21−29] Nevertheless, the widespread application of DI techniques to routine chemical analyses in general chemical and biochemical laboratories is hindered by (1) the requirement of a high-vacuum apparatus for DI operation, (2) poor sensitivity for the detection of volatile or semivolatile compounds, (3) interference from the matrix, particularly in lower mass ranges, and (4) relatively poor reproducibility in quantitative analyses.

There are a number of potential advantages to coupling TLC to MS through an atmospheric pressure spray ionization source, such as an electrospray ionization (ESI) system, including the absence of a postseparation sample preparation procedure prior to analysis (e.g., application of a matrix), a lessening of low-mass chemical noise (in large part caused by the MALDI matrix), the ability to use a variety of sizes of commercially available plates (eliminating vacuum system constraints on sample size), and the benefits of compound-specific ionization afforded by ESI over MALDI.[30−32] Nevertheless, the on-line linking of TLC with ESI-MS has remained particular difficult, mainly because of incompatibility between the requirement of introducing a dynamic liquid-phase sample into ESI-MS and the static nature of TLC. To date, only a few interfaces have been devised to connect TLC with ESI-MS to perform separation and on-line characterization of unknown mixtures.[33−35] Van Berkel and co-workers introduced a novel TLC-ESI-MS system that exploited a surface sampling probe-to-TLC plate liquid microjunction and a self-aspirating ES emitter for the direct detection of small organic compounds on TLC plates.[36−38] Luftmann developed a plunger-based extraction device for TLC-ESI-MS.[39] We have employed a direct electrospray probe to generate analyte ions directly from the end of a TLC plate.[40] Recently, Van Berkel's group applied desorption electrospray ionization (DESI), which Cooks et al. introduced for the analysis of analytes on solids, to couple TLC with MS.[41]

Electrospray-assisted laser desorption/ionization (ELDI), a new ionization method that combines some of the features of ESI and LD, allows the direct, sensitive, and rapid characterization of small organic and large biological compounds in solids under ambient conditions.[42,43] Tedious postseparation and sample pretreatment procedures are often avoided when using this technique for direct solid sample analysis. The ionization processes of ELDI are suggested to be similar to those of fused-droplet electrospray ionization (FD-ESI, or two-step electrospray ionization).[44−46] In an FD-ESI source, gaseous analyte molecules or neutral droplets containing the analyte molecules are conducted to the tip of an electrosprayer by nitrogen, where the analyte molecules are ionized through fusion or reactions with the charged species (including charged solvent droplets, hydronium ions, or protonated solvent species) in the ESI plume. One of the advantages of using FD-ESI for sample analysis is that the ionization and nebulization processes are separate events; this feature provides independent control over the conditions of the sample solution and the composition of the ESI solvent. By varying the methods of introducing the sample into a FD-ESI source, a number of unique applications have been demonstrated for the analyses of liquid, gas, and solid samples.[42−46,47] In essence, the ELDI source uses laser irradiation to produce gaseous analyte molecules; the desorbed analyte molecules then join into the electrospray plume, where they are postionized through ESI.[42,44]

Because a large amount of energy can be introduced to the analyte through a laser pulse, analytes on solid surfaces are often efficiently desorbed through ELDI. In addition, the innately high spatial resolution and scanning capability of the laser beam makes ELDI a useful technique for the rapid and continuous characterization of chemical entities directly from the surfaces of TLC plates. Ionization of the desorbed molecules through ESI processes may also greatly increase the mass accuracy of the detected ions (relative to MALDI). In this paper, we describe the interfacing of ELDI-MS with TLC for the characterization of various organic compounds (FD&C dyes, amines, extracts of a drug tablet) directly from TLC plates.

EXPERIMENTAL SECTION

Materials. Methanol and acetone (HPLC grade) were purchased from Merck (Darmstadt, Germany). Ammonium acetate (97.0%) and acetic acid (GC grade) were obtained from J. T. Baker (Phillipsburg, NJ). FD&C Red no. 3 (erythrosine B, CAS No. 16423−68−0), FD&C Blue no. 1 (erioglaucine, CAS No. 2650−18−2), FD&C Green no. 3 (fast green FCF, CAS No. 2353−45−9), 2,2′-diaminodiethylamine, 3-quinolinamine, and 2-acetylaniline were purchased from Sigma and Aldrich (Milwaukee, WI). Anticold drug tablets (Taiwan Parton Co., Kaohsiung, Taiwan)

(20) Gusev, A. I.; Proctor, A.; Rabinovich, Y. I.; Hercules, D. M. *Anal. Chem.* **1995**, *67*, 1805−1814.
(21) Gusev, A. I.; Proctor, A.; Rabinovich, Y. I.; Hercules, D. M. *Anal. Chem.* **1995**, *67*, 4565−4570.
(22) Mehl, J. T.; Gusev, A. I.; Hercules, D. M. *Chromatographia* **1997**, *46*, 358−364.
(23) Isbell, D. T.; Gusev, A. I.; Taranenko, N. I.; Chen, C. H.; Hercules, D. M. *Fresenius' J. Anal. Chem.* **1999**, *365*, 625−630.
(24) Mehl, J. T.; Hercules, D. M. *Anal. Chem.* **2000**, *72*, 68−73.
(25) Mowthorpe, S.; Clench, M. R.; Cricelius, A.; Richards, D. S.; Parr, V.; Tetler, L. W. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 264−270.
(26) Crecelius, A.; Clench, M. R.; Richards, D. S.; Mather, J.; Parr, V. *J. Planar Chromatogr.* **2000**, *13*, 76−81.
(27) Crecelius, A.; Clench, M. R.; Richards, D. S.; Evason, D.; Parr, V. *J. Chromatogr. Sci.* **2002**, *40*, 614−620.
(28) Crecelius, A.; Clench, M. R.; Richards, D. S.; Parr, V. *J. Chromatogr., A* **2002**, *958*, 249−260.
(29) Crecelius, A.; Clench, M. R.; Richards, D. S. *LCGC Eur.* **2003**, *16*, 225−229.
(30) Doroshenko, V. M.; Laiko, V. V.; Taranenko, N. I.; Berkout, V. D.; Lee, H. S. *Int. J. Mass Spectrom.* **2002**, *221*, 39−58.
(31) Moyer, S. C.; Cotter, R. J. *Anal. Chem.* **2002**, *74*, 469A-476A.
(32) *Electrospray Ionization Mass Spectrometry: Fundamentals, Instrumentation and Applications*; Cole, R. B., Ed; John Wiley: New York, 1997.
(33) Chai, W.; Leteux, C.; Lawson, A. M.; Stoll, M. S. *Anal. Chem.* **2003**, *75*, 118−125.
(34) Aranda, M.; Morlock, G. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 1297−1303.
(35) Van Berkel, G. J.; Llave, J. L.; De Apadoca, M. F.; Ford, M. J. *Anal. Chem.* **2004**, *76*, 479−482.
(36) Van Berkel, G. J.; Sanchez, A. D.; Quirke, J. M. E. *Anal. Chem.* **2002**, *74*, 6216−6223.
(37) Ford, M. J.; Van Berkel, G. J. *Rapid. Commun. Mass Spectrom.* **2004**, *18*, 1303−1309.
(38) Ford, M. J.; Kertesz, V.; Van Berkel, G. J. *J. Mass Spectrom.* **2005**, *40*, 866−875.
(39) Luftman, H *Anal. Bioanal. Chem.* **2004**, *378*, 964−968.
(40) Hsu, F. L.; Chen, C. H.; Yuan, C. H.; Shiea, J. *Anal. Chem.* **2003**, *75*, 2493−2498.
(41) Van, Berkel, G. J.; Ford, M. J.; Deibel, M. A. *Anal. Chem.* **2005**, *77*, 1207−1215.
(42) Shiea, J.; Huang, M. Z.; Hsu, H. J.; Lee, C. Y.; Yuan, C. H.; Beech, I.; Sunner, J. *Rapid. Commun. Mass Spectrom.* **2005**, *19*, 3701−3704.
(43) Huang, M. Z.; Hsu, H. J.; Lee, J. Y.; Jeng, J.; Shiea, J. *J. Proteome Res.* **2006**, *5*, 1107−1116.

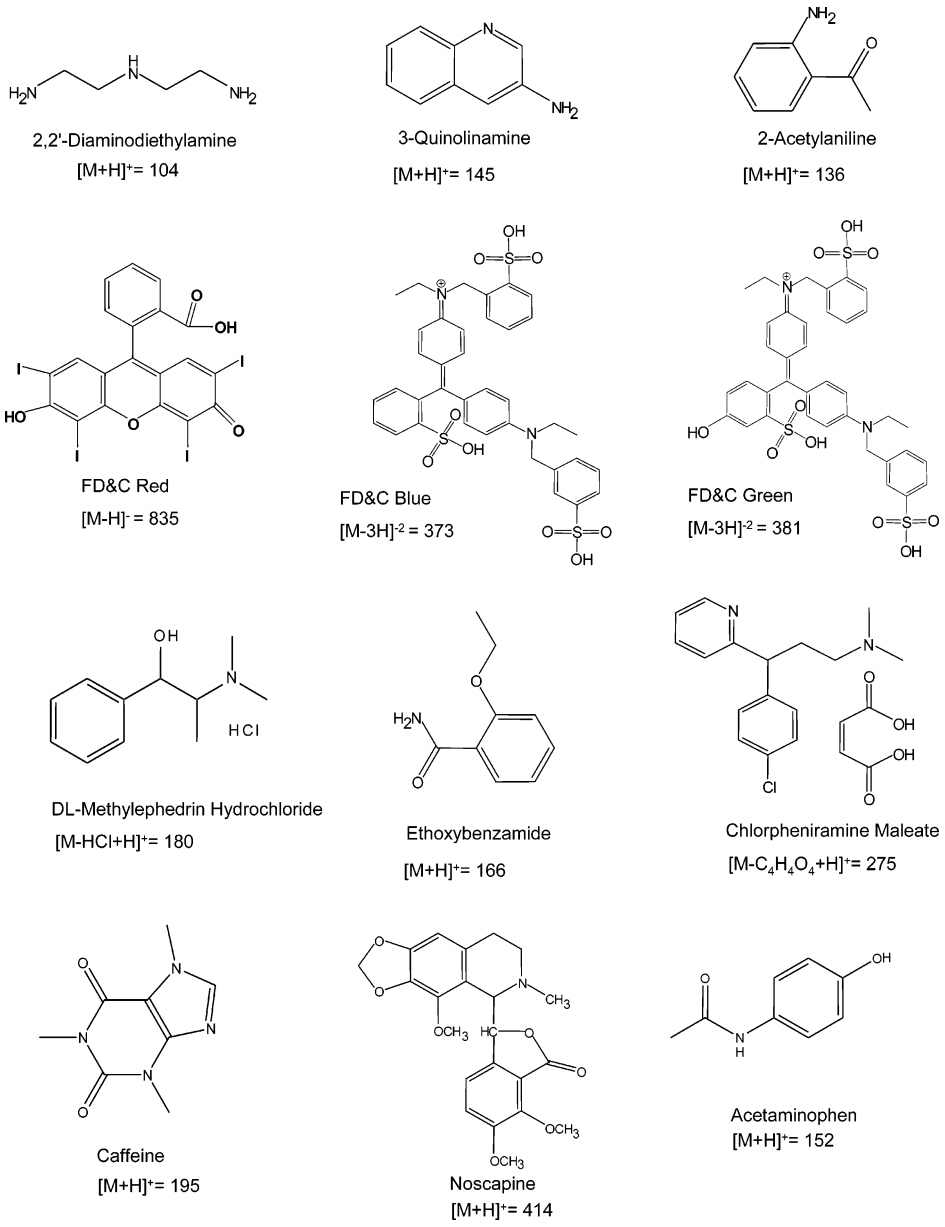

Figure 1. Chemical compounds analyzed in this study.

containing DL-methylephedrin hydrochloride, caffeine, ethoxybenzamide, chlorpheniramine maleate, noscapine, and acetaminophen were purchased from a local pharmacy. Figure 1 displays the chemical structures of the analytes used in this study.

TLC Separation. A methanol solution containing a mixture of three FD&C dyes was spotted in 0.2-μL aliquots at the center of a reversed-phase C_{18} TLC plate (RP-18F, Merck; 0.2 mm thick, 0.5 cm wide, and 3 cm long). The concentrations of the dyes in the sample solution were 18.5 (FD&C Red dye), 17.0 (FD&C Green dye), and 16.6 ng/μL (FD&C Blue dye). A solution of 500 mM ammonium aqueous acetone (70/30, v/v) was used as the mobile phase for developing the dyes on the TLC plate. After development, most of the sample spots were visible. The TLC plate was air-dried or dried in an oven (110 °C) for 15 min prior to further ELDI/MS analysis. Photographs of the developed plates were taken using a digital camera (LUMIX, Panasonic Japan) under white light.

A methanol solution containing mixtures of amines was spotted in 0.2-μL aliquots at the center of a normal-phase silica TLC plate (Silica gel 60, Merck; 0.5 mm thick, 0.5 cm wide, and 3 cm long). The concentrations of the amines in the sample solution were 2.08 (diaminodiethylamine), 2.9 (3-quinolinamine), and 2.7 μg/μL (2-acetylaniline). A solution containing ethyl acetate, acetic acid, and dichloromethane (98:1:1, by volume) was used as the mobile phase for developing the amines on the TLC plate.

A normal-phase silica gel TLC plate was also used to separate the compounds extracted (by 5 mL of MeOH/ethyl acetate, 50:50 by volume) from a commercial anti-cold tablet. The extracted solution was spotted on the TLC plates in 0.2-μL aliquots. The plate was then developed using the solution of ethylacetate, acetic acid, and CH_2Cl_2 (98:1:1, by volume).

TLC/ELDI-MS System. Figure 2 displays a schematic illustration of the ELDI-MS approach to desorption and ionization of organic compounds on a TLC plate. The best distance and

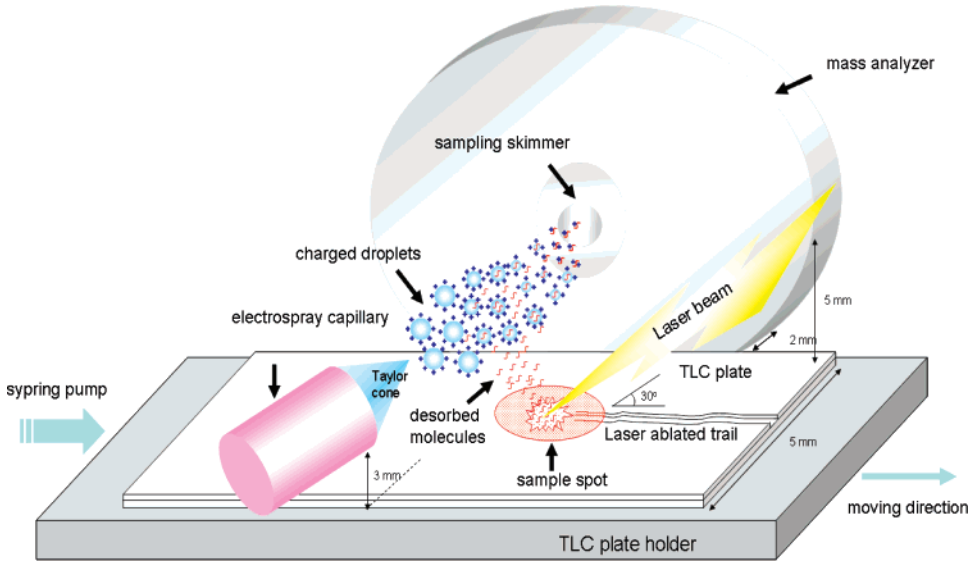

Figure 2. Schematic illustration of the ELDI-MS approach to the desorption and ionization of organic compounds separated on a TLC plate.

location between the electrosprayer and the TLC plate (as shown in Figure 2) were predetermined by adjusting the XYZ stage until a maximum standard ion signal (FD&C red dye) was obtained. After development, the TLC plate was placed on an acrylic sample holder set in front of the sampling skimmer of an ion trap mass analyzer. The sample holder was then moved slowly from left to right using a syringe pump operated at a speed of ~4 mm/min. A pulsed nitrogen laser ablated a trail, barely visible to the naked eye, as it traversed the center of the TLC plate. The analyte spots on the TLC plate were then exposed to irradiation by a pulsed nitrogen laser having a wavelength of 337 nm, operated at 10 Hz (controlled by a sweep function generator), a pulsed energy of ~150 μJ, and length of 4 ns. The laser beam (spot size, ~100 μm × 150 μm) was focused through an objective lens. The desorbed sample molecules then entered into an ESI plume and were postionized through their reactions with the charged species generated by electrospray.

The electrospray solutions comprised methanol and water (1:1, v/v) with 0.1% acetic acid for positive ion detection and methanol and water solution (1:1, v/v) alone for negative ion detection. The ESI solution was delivered through a fused-silica capillary (100-μm i.d.) at a flow rate of 2 μL/min. A nebulizing gas, commonly used in conventional ESI sources, was not used during ELDI.[40,42−47] The ESI plume was directed toward the ion sampling orifice (i.e., parallel to the TLC plate). The resulting analyte ions were sampled into the ion trap mass analyzer (Bruker Dalton Esquire 3000+) through the ion sampling capillary. The electrospray needle and the sample plate potentials were both held at 0 V (grounded); the sampling cone voltage in the ion trap mass spectrometer (Bruker Daltonics, Esquire 3000+) was maintained at −4.5 kV. The ions generated in the TLC/ELDI source were directed toward the sampling skimmer of the ion trap mass analyzer and the mass spectra were recorded at a scan rate of ~2 scans/s.

RESULTS AND DISCUSSION

Although it is not demonstrated in this study, the results of our previous studies indicate that no analyte ion signal was detected in the absence of laser irradiation or ESI plume, and because the ESI plume is aligned parallel to the sample plate, we believe that DESI-like ion formation processes do not occur during ELDI.[48]

The detection limits for organic compounds on TLC plates analyzed by ELDI-MS were estimated using the solutions containing different concentrations of the FD&C Red dye standard (from 10^{-3} to 10^{-7} M). Each standard solution (2 μL) was deposited on a reversed-phase C_{18} TLC plate; after air-drying, the plate was scanned lineally with the UV laser of an ELDI-MS system operated in negative-ion mode. The molecular ion of the FD&C Red dye standard (m/z 835, $(M - H)^-$) was detected only when the concentration of F&C Red dye in the solution was higher than 10^{-6} M (Figure 3a−d). We observed no degraded ions from FD&C red dye on the ELDI mass spectra. This is similar to that by conventional ESI analysis (data not shown). We concluded that the detection limit of the TLC/ELDI-MS approach was ~10^{-6} M. Figure 3f shows the ion intensity of m/z 835 (the ion peaks were not smoothed) detected from the spots containing different concentrations of FD&C Red dye versus scan time. A calibration curve based on the results in Figure 3f was shown in Figure 3g, and a linear relationship was obtained between 10^{-3} and 10^{-6} M. Although the results of duplicate quantitative analysis are similar, we still suggested that only semiquantitative analysis should be perform. This is because the ion intensity of an analyte on TLC plate will be varied by the laser power, laser focusing size, thickness and composition of the silica gel, and diffusion coefficient of the analyte on the TLC plate during ELDI/MS analysis. To confirm the structure of the analyte, we performed an MS/MS analysis of the FD&C Red dye's molecular ion (m/z 835, $[M - H]^-$). Three negative daughter ions ($[M - COOH]^- = m/z$ 791; $[M - COOH - I]^- = m/z$ 633; and $[M - COOH - I_2]^- = m/z$ 537) appeared in the resulting MS/MS spectrum (Figure 3e).

(44) Shiea, J.; Lee, C. C.; Chang, D. Y.; Jeng, J. *J. Mass Spectrom.* **2002**, *37*, 115−117.
(45) Shiea, J.; Chang, D. Y.; Lin, C. H.; Jiang, S. J. *Anal. Chem.* **2001**, *73*, 4983−4987.
(46) Shiea, J.; Wang, C. H. *J. Mass Spectrom.* **1997**, *32*, 247−250.
(47) Lee, C. Y.; Shiea, J. *Anal. Chem.* **1998**, *70*, 2757−2761.
(48) Huang, M. Z.; Hsu, H. J.; Wu, C. I.; Lin, S. Y.; Ma, Y. L.; Cheng, T. L.; Shiea, J. *Rapid. Commun. Mass Spectrom.* **2007**, *21*, 1767−1775.

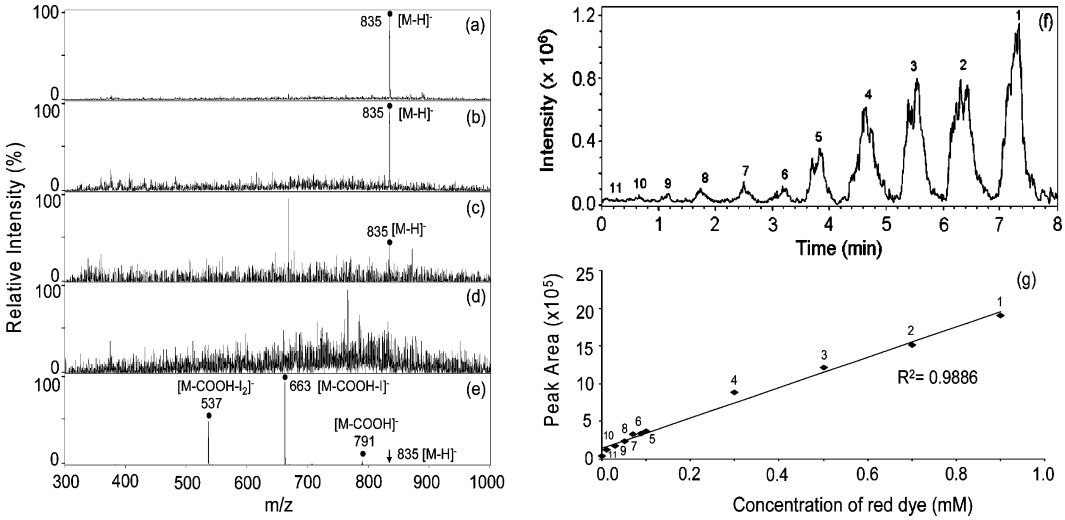

Figure 3. Negative-ion ELDI mass spectra recorded from four sample spots deposited on a reversed-phase C_{18} TLC plate. The sample solutions (1 μL) were FD&C Red dye standards (*m*/*z* 835 for $[M - H]^-$) dissolved in water at concentrations of (a) 10^{-4}, (b) 10^{-5}, (c) 10^{-6}, and (d) 10^{-7} M. The MS/MS spectrum of the parent ion of FD&C Red dye (*m*/*z* 835) is presented in (e). (f) The ion intensity (*m*/*z* 835) of the sample spots on the plate vs scanning time. (g) The calibration curve based on the peak area of *m*/*z* 835 in (f) vs the concentration of the sample spots.

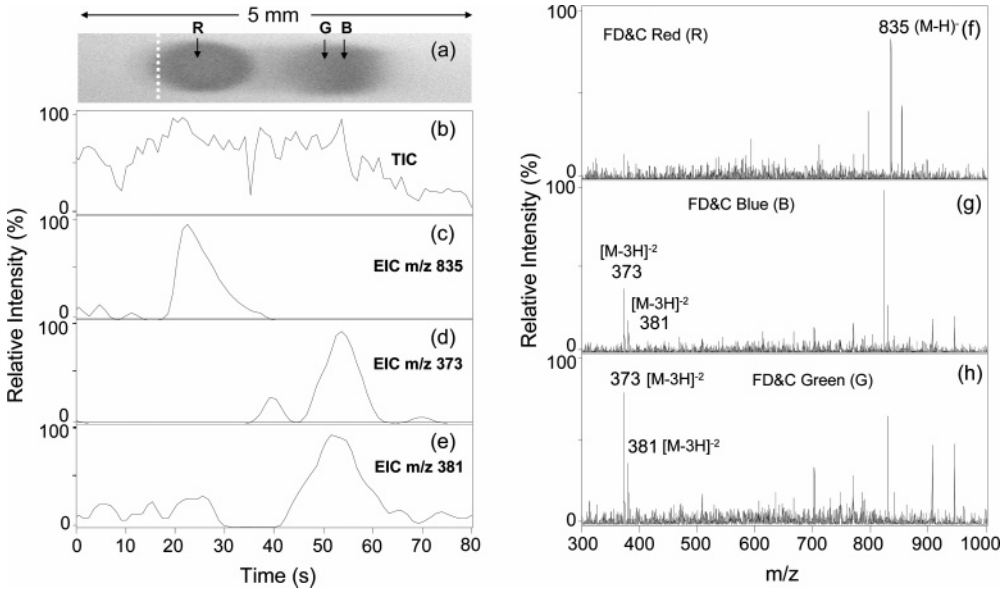

Figure 4. (a) Photographic image of the separation of a mixture of three FD&C dyes on a partial reversed-phase C_{18} TLC plate. (b) Total ion chromatogram and (c−e) extracted ion chromatograms for the (c) singly charged red dye ion (R, *m*/*z* 835), (d) doubly charged blue dye ion (B, *m*/*z* 373), and (e) doubly charged green dye ion (G, *m*/*z* 381) obtained from full-scan negative-ion-mode data acquired by scanning the developed lane indicated in (a). (f−h) Background-subtracted, averaged mass spectra (negative-ion mode) recorded at locations in the chromatogram corresponding to the respective spot positions on the TLC plate: (f) red dye, (g) blue dye, and (h) green dye.

This result is similar to that obtained using a conventional ESI-MS/MS system (data not shown).

A reversed-phase C_{18} TLC plate was used to separate a mixture of three FD&C dyes (FD&C dyes at a concentration of 10^{-5} M for each dye). Figure 4a displays a photograph of the developed partial TLC plate. The blue and green dyes were not totally resolved on the TLC plate; The R_f values of the dyes were 0.03, 0.17, and 0.18 for the red, green, and blue dyes, respectively.

Figure 4b presents the reconstructed total ion chromatogram of the ELDI-MS analysis. Figure 4c−e display the extracted ion chromatograms of the red, blue, and green dyes acquired with the ion trap mass spectrometer set to collect negative-ion full-scan mass spectra during the surface scan of the TLC plate. Figure 4f−h presents the mass spectra recorded at each sample spot on the plate. The peaks above *m*/*z* 900 in Figure 4g and h may be due to impurities in the FD&C Blue and Green dyes. Each dye standard was detected as either a singly (*m*/*z* 835 for red dye) or doubly charged (*m*/*z* 373 for blue dye; *m*/*z* 381 for green dye) molecular anion. Since green dye (G) and blue dye (B) both contain a positively charged tertiary amine group, G-3H and B-3H then have two negative charges (see Figure 1). We observed some ion suppression effects when the spot containing both FD&C Blue and Green dyes was analyzed (by comparing the ion intensity of FD&C Green and Blue dyes in Figure 4g and h). This also happened when conventional ESI was used to analyze the solution containing both compounds.

A normal-phase silica gel TLC plate was used to separate a mixture of 2,2′-diaminodiethylamine, 3-quinolinamine, and 2-acety-

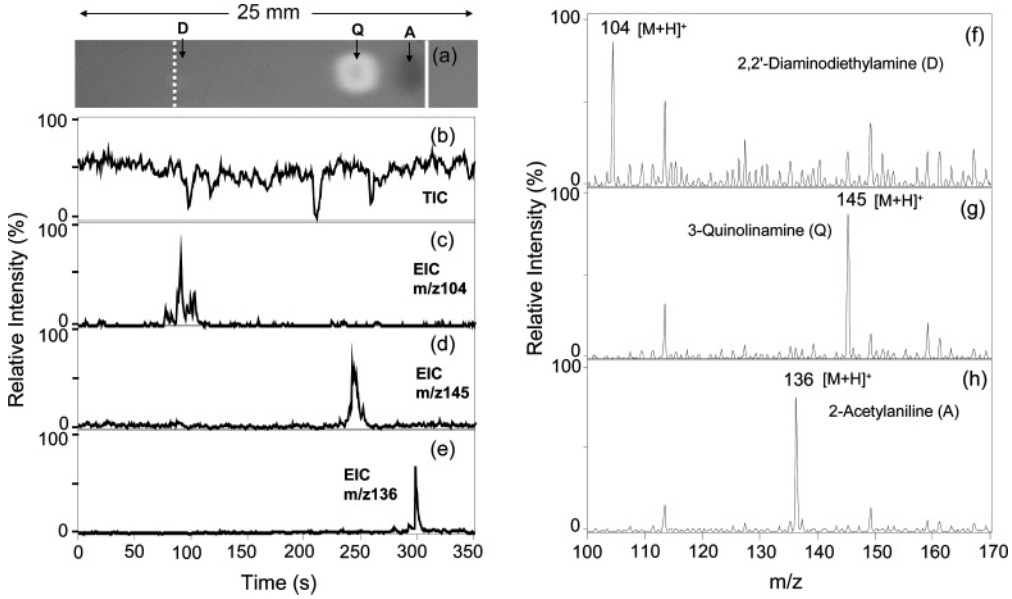

Figure 5. (a) Photographic image of a separated mixture of three amines (20 mM; 0.2 μL) on a silica gel TLC plate. (b) Total ion chromatogram and (c−e) extracted ion chromatograms for (c) 2,2′-diaminodiethylamine (D, *m*/*z* 104), (d) 3-quinolinamine (Q, *m*/*z* 145), and (e) 2-acetylaniline (A, *m*/*z* 136) obtained from full-scan positive-ion-mode data acquired by scanning the developed lane indicated in (a). (f−h) Background-subtracted, averaged positive-ion mass spectra recorded at the locations in the chromatogram corresponding to the respective spot positions on the TLC plate: (f) 2,2′-diaminodiethylamine, (g) 3-quinolinamine, and (h) 2-acetylaniline.

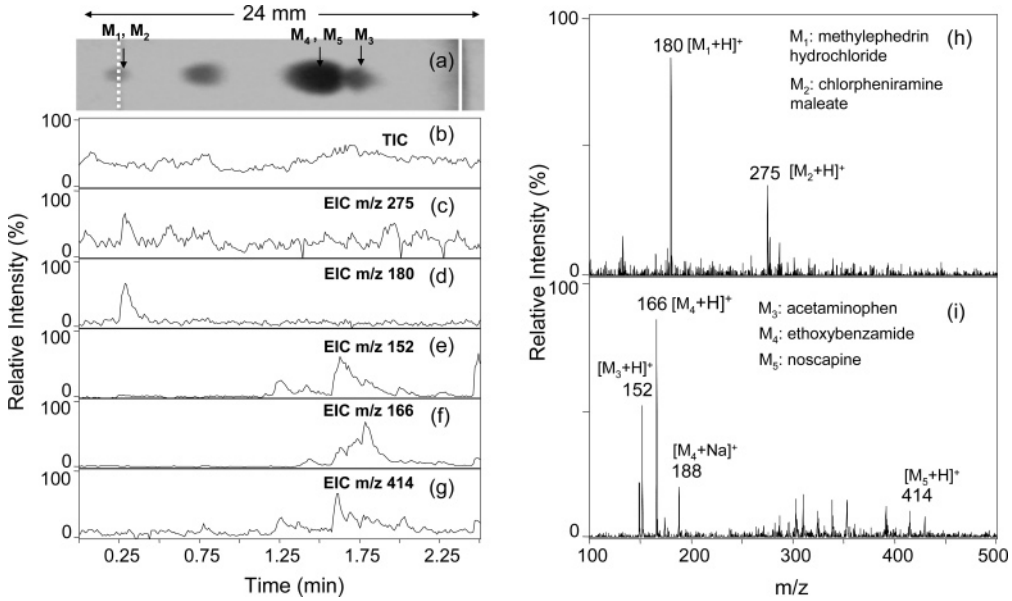

Figure 6. (a) Photographic image of a normal-phase TLC plate after separation of the active ingredients extracted from an anti-cold tablet. (b) Total ion chromatogram and (c−g) extracted ion chromatograms for (c) chlorpheniramine maleate (M2, *m*/*z* 275) (d) methylephedrin hydrochloride (M1, *m*/*z* 180), (e) ethoxybenzamide (M4, *m*/*z* 152), (f) acetaminophen (M3, *m*/*z* 166), and (g) noscapine (M5, *m*/*z* 414) obtained from full-scan positive-ion-mode data acquired by scanning the developed lane indicated in (a). (h, i) Background-subtracted, averaged positive-ion mass spectra recorded at locations in the chromatogram corresponding to the respective spot positions on the TLC plate: (h) methylephedrin hydrochloride and chlorpheniramine maleate (i) acetaminophen, ethoxybenzamide, and noscapine.

laniline (0.2 μL each of 20 mM sample solution). Figure 5a displays a photograph of the TLC separation of these compounds. The R_f values by the spots of 3-quinolinamine and 2-acetylaniline on the TLC plate were 0.75 and 0.97, respectively. Because of its high polarity and strong interactions with the hydroxyl groups on the silica stationary phase, the 2,2′-diaminodiethylamine spot did not move at all during development ($R_f = 0$). Figure 5b presents the total ion chromatogram obtained when the surface of the TLC plate was scanned and analyzed by the ELDI-MS system in positive-ion mode. During the ELDI analysis, the electrosprayer continuously generated charged species from the ESI solvents. Therefore, most of the ion current detected by ELDI is from the solvent ions (including those from methanol, water, and acetic acid). This is the reason why distinct analyte peaks were not detected, since the concentration of the analyte ions is usually lower than that of the solvent ions. Panels c−e in Figure 5 are the extracted ion chromatograms corresponding to 2,2′-diaminodiethylamine (m/z 104), 3-quinolinamine (m/z 145), and 2-acetylaniline (m/z 136), respectively. Panels f−h in Figure 5 display the ELDI mass spectra of the respective analytes.

Although the main chemical components in the tablet can be directly detected by irradiating the surface of the table with a laser, extraction and separation may still be required for a tablet with multiple components.[48] In this study, the chemicals in an anti-cold tablet were extracted and analyzed using the TLC/ELDI-MS system. The active ingredients of the tablet (0.5 g) included DL-methylephedrin hydrochloride (10 mg), caffeine (30 mg), ethoxybenzamide (120 mg), chlorpheniramine maleate (2.5 mg), noscapine (10 mg), and acetaminophen (200 mg). A normal-phase silica gel TLC plate was used to separate 0.2-μL aliquots of the solution (5 mL of MeOH/ethyl acetate, 50:50 by volume) extracted from the anti-cold tablet. Figure 6a displays the photograph of the TLC plate obtained after separation of these components. Figure 6b presents the total ion chromatogram acquired when using the ion trap mass spectrometer to collect positive-ion full-scan mass spectra during the pulsed nitrogen laser's surface scan of the TLC plate. Since we were unable to obtain the standards of most active ingredients in the tablet, the identification of each active ingredient was then based on the m/z of the detected molecular ions. Panels c−g in Figure 6 display extracted molecular ion chromatograms of the active ingredients in the tablet, and panels h and i in Figure 6 present their respective mass spectra. Several ion peaks were detected between m/z 250 and 375 in Figure 6i; we suspected that the peaks might be from the impurities in the tablet or adducted or cluster analyte ions. Although caffeine was labeled as the active ingredient in the tablet, we did not detect its molecular ion signal. This might due to low concentration of caffeine in the tablet (30 mg/500 mg), high volatility, and low gas-phase basicity.

CONCLUSION

With this study, we have demonstrated that ELDI-MS can be used to directly characterize molecules separated on either reversed-phase C_{18} or normal-phase silica gel TLC plates when employing MS or MS/MS in positive- or negative-ion mode. Since the use of organic or inorganic matrixes is unnecessary for ELDI analysis, this feature greatly simplifies the sample preparation procedure. Because a large amount of energy can be transferred to the analyte from the UV laser pulse, the molecules on solids having hard surfaces (such as TLC plates) can be efficiently desorbed when using ELDI. The innately high spatial resolution and scanning capability of the laser beam makes ELDI a useful technique for the rapid and continuous characterization of molecules adsorbed onto the surfaces of TLC plates. The desorption and ionization processes of ELDI are performed at atmospheric pressure, and thus, it is readily compatible with almost all mass analyzers.

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