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Thin-Layer Chromatography/Desorption Electrospray Ionization Mass Spectrometry: Investigation of Goldenseal Alkaloids

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Desorption electrospray ionization mass spectrometry was investigated as a means to qualitatively identify and to quantify analytes directly from developed normal-phase thin-layer chromatography plates. The atmospheric sampling capillary of a commercial ion trap mass spectrometer was extended to permit sampling and ionization of analytes in bands separated on intact TLC plates (up to 10 cm × 10 cm). A surface positioning software package and the appropriate hardware enabled computer-controlled surface scanning along the length of development lanes or at fixed R_f value across the plates versus the stationary desorption electrospray emitter. Goldenseal (*Hydrastis canadensis*) and related alkaloids and commercial dietary supplements were used as standards and samples. Alkaloid standards and samples were spotted and separated on aluminum- or glass-backed plates using established literature methods. The mass spectral signal levels as a function of desorption spray solvent were investigated with acetonitrile proving superior to methanol. The detection levels (∼5 ng each or 14−28 pmol) in mass spectral full-scan mode were determined statistically from the calibration curves (2.5−100 pmol) for the standards berberine, palmatine, and hydrastinine spotted as a mixture and separated on the plates. Qualitative screening of the major alkaloids present in six different over-the-counter "goldenseal" dietary supplements was accomplished by obtaining full-scan mass spectra during surface scans along the development lane in the direction of increasing R_f value. In one sample, alkaloids were detected that strongly suggested the presence of at least one additional herb undeclared on the product label. These same data indicated the misidentification of one of the alkaloids in the TLC literature. Quantities of the alkaloids present in two of the samples determined using the mass spectral data were in reasonable agreement with the label values, indicating the quantitative ability of the method. The advantage of mass spectral measurements in identifying and quantifying materials within overlapping bands and in providing positive identification for even minor species in a mixture was also demonstrated.

Desorption electrospray ionization-mass spectrometry (DESI-MS) provides the means to sample and ionize analytes directly from surfaces at ambient conditions with subsequent mass spectrometric detection.[1−3] The analytes typically amenable to analysis by conventional ES-MS (e.g., ionic, polar, and thermally labile molecules and biomolecules),[4] may also be analyzed by DESI-MS. These basic DESI-MS sampling and ionization capabilities provide the components that are needed to advance instrumentation for determining the spatial distribution of analytes on important surfaces under ambient conditions. Presented with this possibility, we began to explore the use of DESI-MS as a means to investigate analytes separated on thin-layer chromatography (TLC) plates.[5,6] Others are beginning to explore this area as well.[7]

Coupling TLC with mass spectrometric detection pairs a very simple and robust separation methodology with a detector that exhibits the ability to qualitatively identify and detect materials with great selectivity.[8−10] When appropriate standards are available, mass spectrometry can be used to quantitate known materials. A simple, robust, and automated TLC/MS system for the analysis of developed plates that can use intact, off-the-shelf TLC plates, with any of a variety of separation phases and without postdevelopment processing, has long been desired, but not realized.[11−13] The currently most popular TLC/MS coupling uses matrix-assisted laser desorption/ionization (MALDI), but no commercial instrumentation exists specifically designed for TLC/MS. Spray ionization techniques have also been investigated. Some of the more successful couplings have made use of "liquid junction"—(with ES-MS[14−17] or atmospheric pressure chemical ionization

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(1) Takáts, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G. *Science* **2004**, *306*, 471−473.
(2) Takáts, Z.; Wiseman, J. M.; Cooks, R. G. *J. Mass Spectrom.* **2005**, *40*, 1261−1275.
(3) Cooks, R. G.; Ouyang, Z.; Takáts, Z.; Wiseman, J. M. *Science* **2006**, *311*, 1566−1570.
(4) Cech, N. B.; Enke, C. G. *Mass Spectrom. Rev.* **2001**, *20*, 362−387.
(5) Van Berkel, G. J.; Ford, M. J.; Deibel, M. A. *Anal. Chem.* **2005**, *77*, 1207−1215.
(6) Van Berkel, G. J.; Kertesz, V. *Anal. Chem.* **2006**, *78*, 4938−4944.
(7) Kaupplia, T.; Talaty, N.; Salo, P. K.; Kotiaho, T.; Kostiainen, R.; Cooks, R. G. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2143−2150.
(8) Siouffi, A..*Sep. Purif. Rev.* **2005**, *34*, 155−180.
(9) Poole, C. *J. Chromatogr., A* **2003**, *1000*, 963−984.
(10) Poole, C. *J. Chromatogr., A* **1999**, *856*, 399−427.
(11) Wilson, I. D. *J. Chromatogr., A* **1999**, *856*, 429−442.
(12) Gusev, A. I. *Fresenius J. Anal. Chem.* **2000**, *366*, 691−700.
(13) Busch, K. L. Planar Separations and Mass Spectrometric Detection. *J. Planar Chromatogr.* **2004**, *17*, 398−403.
(14) Van Berkel, G. J.; Sanchez, A. D.; Quirke, J. M. E. *Anal. Chem.* **2002**, *74*, 6216−6223.

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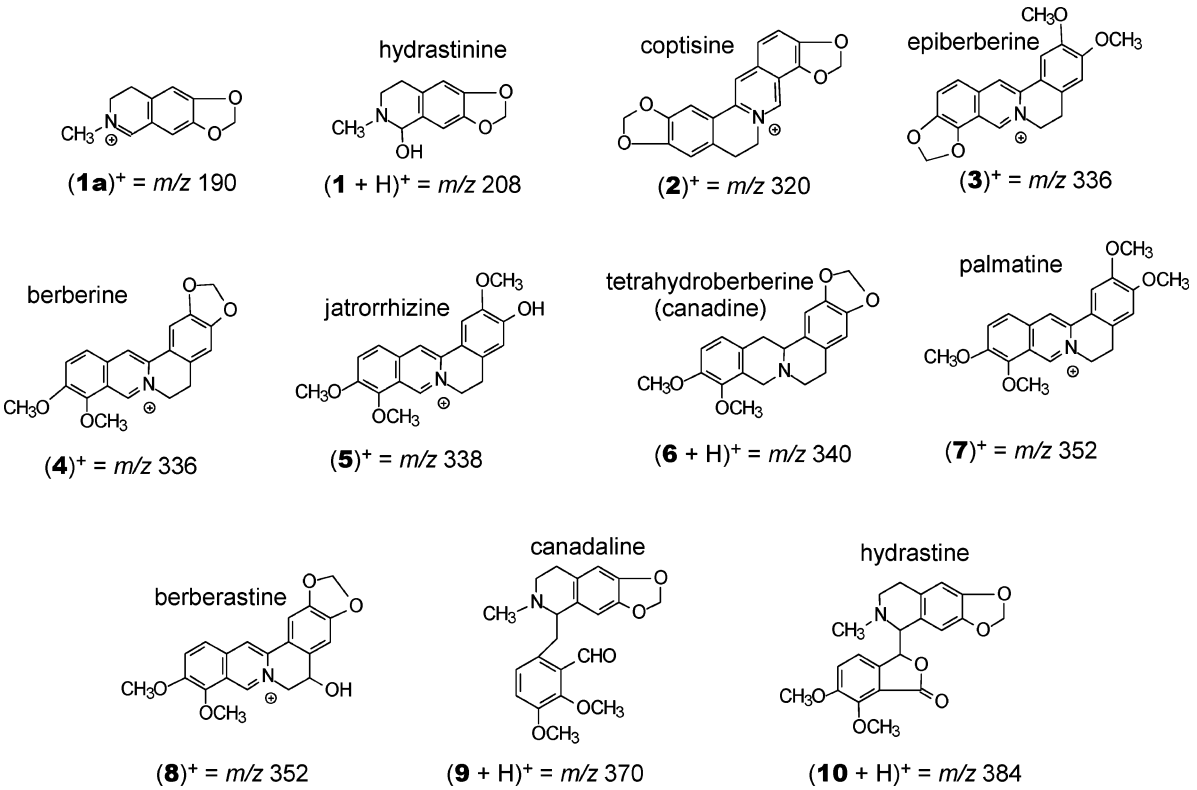

Figure 1. Structure and mass-to-charge ratio for goldenseal and related alkaloids.

(APCI)-MS[18]) or "sealing"—surface sampling probe approaches (with ES-MS[19,20] or APCI-MS[21]). All of these approaches have been used to garner useful analytical information, but each has been limited to some degree by the need for postdevelopment processing of the plates (e.g., matrix application for MALDI), the need for specialized plates (e.g., size limits to fit the vacuum chamber in MALDI), limits to the phases that can be analyzed (e.g., hydrophobic reversed phase for the liquid junction probe), or manual intervention in the analysis (sealing−surface sampling probe), among other limitations.

As demonstrated by the results in our prior reports,[5,6] using DESI-MS as an interface may overcome many of the limitations of prior approaches to TLC/MS. In our first report, DESI-MS with computer-controlled plate movement was used to accomplish the scanning of a single development lane on a TLC plate. Various dyes and pharmaceuticals were separated and analyzed on both normal- and reversed-phase (hydrophobic and wettable) plates. We expanded on that work through instrument and hardware modifications to demonstrate computer-controlled spot sampling of select bands on a plate, scanning of multiple development lanes on a plate, and imaging of analyte bands in a development lane.[22] Rhodamine dyes separated on hydrophobic reversed-phase C8 plates were used in those studies. Those two reports showed that TLC/DESI-MS could be a completely hands-off analysis and that the analysis of spots or lanes could be accomplished in a relatively rapid fashion. Furthermore, one could examine intact and unmodified commercial plates employing any one of the common TLC separation phases under ambient conditions.

In the present report, we concentrate on the quantitative determination of materials separated on the plate as well as the qualitative identification, confirmation, and comparison of the components in real samples using DESI-MS. For several reasons, over-the-counter goldenseal dietary supplements, and the alkaloids therein and related alkaloids, were chosen as standards and samples for study (see chemical structures in Figure 1). First, as in ES-MS,[4] analytes with a "fixed charge" appear to work best in DESI-MS. The goldenseal alkaloids provide the opportunity to examine both fixed-charge ions and neutral bases that must be protonated to be detected. This allowed an initial examination of the differences in detection among these different ion types. Second, established methods for separation of goldenseal alkaloids employ normal-phase plates, which is the most commonly used separation phase.[23] A highly practical TLC/MS methodology must be able to use this separation phase. Third, one of the major application areas for TLC today is the analysis of botanicals and dietary supplements for identification and quality control.[24] Goldenseal supplements are among the most popular supplements sold, and numerous commercial brands of different formulation are available over the counter.[25,26]

(15) Ford, M. J.; Van Berkel, G. J. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 1303−1309.
(16) Ford, M. J.; Kertesz, V.; Van Berkel, G. J. *J. Mass Spectrom.* **2005**, *40*, 866−875.
(17) Ford, M. J.; Deibel, M. A.; Tomkins, B. A.; Van Berkel, G. J. *Anal. Chem.* **2005**, *77*, 4385−4389.
(18) Asano, K. G.; Ford, M. J.; Tomkins, B. A.; Van Berkel, G. J. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 2305−2312.
(19) Luftmann, H. *Anal. Bioanal. Chem.* **2004**, *378*, 964−968.
(20) Jautz, U.; Morlock, G. *J. Chromatogr., A* **2006**, *1128*, 244−250.
(21) Prosek, M.; Milivojevic, L.; Krizman, M.; Fir, M. *J. Planar Chromatogr.* **2004**, *17*, 420−423.
(22) Kertesz, V.; Ford, M. J.; Van Berkel, G. J. *Anal. Chem.* **2005**, *77*, 7183−7189.
(23) Reich, E.; Schibli, A. *LC-GC North Am.* **2005**, *23* (5), 458−469.
(24) Reich, E.; Blatter, A. *Inside Lab. Manage.* **2004**, (May/June), 14−18.

Table 1. Summary of Over the Counter Goldenseal Dietary Supplements Examined

brand name	product description	serving description	weight per serving	declared alkaloid content
GNC Herbal Plus Full Spectrum Goldenseal Root Herbal Supplement	goldenseal root extract *(H. canadensis)*; vitamin C ;soybean oil; marine microalgae oil	softgel capsule	125 mg goldenseal root extract	6.2 mg hydrastine (5% w/w of each capsule)
Nature's Resource Goldenseal	goldenseal root (*H. canadensis)*	gelatin capsule with dried ground root	535 mg goldenseal root	berberine, 13.4 mg; hydrastine, 10.7 mg
NOW Goldenseal Root	goldenseal powder (*H. canadensis)* (root)	capsule with dried ground root	500 mg goldenseal powder	not listed
SOLARAY Goldenseal Root Extract	goldenseal *(H. canadensis)* (root extract); Goldenseal *(H. canadensis)* (root)	gelatin capsule with dried ground root	250 mg. Ingredients specifically list 250 mg root extract and 65 mg root	10% per capsule, or 25 mg from root extract
SOLGAR Goldenseal Root Complex Extract	standardized goldenseal (*H. canadensis*)/ goldthread extract (*C. chinensis)* (root); raw goldenseal powder (root)	vegetable capsule with ground root	450 mg, as 150 mg standardized goldenseal/ goldthread root extract and 300 mg pure goldenseal powder (root)	alkaloids 15 mg (10%) berberine, hydrastine, palmatine, based on the standardized root extract
Walgreens Goldenseal Root	goldenseal root powder *(H. canadensis)*	capsule with dried ground root	500 mg goldenseal root powder	not listed

We report the effect of different desorption solvents on TLC/DESI-MS signal intensity and determine statistically from external calibration curves the detection levels of three alkaloid standards separated on normal-phase plates. In addition, we compare the label values for the alkaloid content in two goldenseal dietary supplements with the alkaloid content determined by TLC/DESI-MS and by fluorescence methods. We also qualitatively screen for the major alkaloids present in six different over-the-counter goldenseal dietary supplements. As a whole, the results presented show that the current TLC/DESI-MS system provides detection levels comparable to routine fluorescence methods (low-nanogram range) and that quantitative analysis using the method of external standards provides values consistent with manufacturer label values. Mass selective detection offers improved selectivity compared to simple fluorescence-based quantitation in the case of overlapping analyte bands on the plate. The advantage of mass spectral measurements in finding materials in overlapping bands and providing positive identification for even minor species in a mixture is also demonstrated.

EXPERIMENTAL SECTION

Chemicals, Reagents, and Standards. Methanol and water were purchased from J. T. Baker (Phillipsburg, NJ), and acetonitrile and ethyl acetate were obtained from Burdick & Jackson (Muskegon, MI). Acetic acid (PPB/Teflon grade, purified by double distillation) and formic acid (≥96% purity) were purchased from Sigma-Aldrich (St. Louis, MO).

Berberine chloride (CAS Registry No. 633−65-8), palmatine chloride hydrate (CAS Registry No. 171869−95-7, (1*S*,9*R*)-(+)-β-hydrastine (CAS Registry No. 29617−43-4), tetrahydroberberine (*syn.* canadine, CAS Registry No. 522−97-4), and hydrastinine hydrochloride (CAS Registry No. 4884−68-8) were purchased from Sigma-Aldrich. Jatrorrhizine (CAS Registry No. 3621−38-3) was purchased from Herb Standard, Inc. (Lemont, IL). All compounds were used as received. Approximately 2−20 mg of each were dissolved in individual 10-mL portions of methanol to form stock solutions, which were used to prepare a mixed standard and diluted further as needed.

Goldenseal Samples. Six different over-the-counter goldenseal dietary supplements were purchased locally (Table 1). According to the products labels, four of these contained 250−500 mg of loose ground "goldenseal root" in capsules, viz., Walgreens (Deerfield, IL), Nature's Resource (Mission Hills, CA), Now Foods (Bloomingdale, IL), and Solaray (Neutraceutical Corp., Park City, UT). Herbal Plus Full Spectrum goldenseal herbal supplement in "hard" capsules, which was purchased from GNC (General Nutrition Corp., Pittsburgh, PA), contained not only goldenseal root extract (125 mg) but also glycerin and marine microalgae oil. Solgar "goldenseal root complex extract", which was obtained from Solgar Vitamin and Herb (Leonia, NJ), contained both loose ground goldenseal (*Hydrastis canadensis*) and the herb goldthread (*Coptis chinensis*) in capsules (450 mg).

Extraction Procedure. The extraction procedure was similar to that recommended in the literature.[25] A portion of dry goldenseal root (250 mg) was weighed into a 10-mL plastic centrifuge tube, followed by 4 mL of a methanol/water (80/20 v/v) mixture. (In the special case of the GNC goldenseal, two hard capsules were broken prior to addition to the centrifuge tube.) The goldenseal and methanol/water mixture was extracted in an ultrasonic bath for 30 min at room temperature and then centrifuged (3100 rpm) for 10 min. The supernatant was diluted to a final volume of 25 mL with methanol. The final solution was filtered if necessary, with a 0.45-μm-porosity nylon syringe filter and then transferred to a precleaned 40-mL vial. The light- and

(25) Upton, R., Ed. *Goldenseal Root, Hydrastis Canadensis: Standards of Analysis, Quality Control, and Therapeutics*; American Herbal Pharmacopoeia: Santa Clara, CA, 2001.

(26) Weber, H. A.; Zart, M. K.; Hodges, A. E.; White, K. D.; Narnes, S. M; Moody, L. A.; Clark, A. P.; Harris, R. K. Overstreet, J. D.; Smith, C. S. *J. AOAC Int.* **2003**, *86*, 476−483.

heat-sensitive extract was stored in a refrigerator (4 °C) when not in use.

TLC. Most of the TLC separations were performed using aluminum sheets (5 × 20 cm) precoated with silica gel 60 F_{254} (layer thickness 0.2 mm), which were purchased from EM Reagents (Darmstadt, Germany, Catalog No. 5534, TLC). Additional work was performed using silica gel 60 (layer thickness 0.2 mm), 10 × 10 cm glass-backed plates (EM Reagents, Catalog No. 5631, HPTLC). All plates were spotted with 1- or 5-μL volumes of standards or extracts using either Drummond Microcaps (Drummond Scientific Co., Broomall, PA) or a conventional manual pipettor and allowed to air-dry. The plates were developed in ascending mode in a glass chamber saturated with the developing solvent (50/10/6/3 by volume ethyl acetate/methanol/formic acid/water, respectively).[25] A VersaDoc model 3000 Imaging System (BioRad Laboratories, Hercules, CA) was used in UV epi-illumination mode (2-s exposure) to obtain images of the TLC plates prior to the TLC/DESI-MS experiments. The images were converted to bitmap format and the band intensities were integrated using the UN-SCAN-IT gel software version 5.1 (Silk Scientific, Orem, UT). Photographs of the developed plates were taken with a Coolpix 990 digital camera (Nikon, Tokyo, Japan) using long-wavelength UV illumination.

Optimization of the ESI-MS Conditions. The mass spectrometer used in this work was a ThermoFinnigan LCQ DecaXP Plus ion trap (Thermo-Finnigan, San Jose, CA) operated using Xcalibur version 1.4 software. Methanolic solutions containing 1.0 or 0.10 pmol/μL (μM) of each standard were electrosprayed in positive ion mode using the ES source supplied with the instrument to identify the mass spectrometric conditions producing the most intense molecular ionic signal for each of the respective standards. Optimum capillary and tube lens voltages varied slightly among standards, so a compromise set of conditions was chosen that produced near-maximum signal levels from all analytes. Mass spectra were acquired with a heated capillary temperature of 250 °C, 4.0-kV spray voltage applied to the DESI emitter, 19-V capillary voltage, and 5-V tube lens offset. Product ion tandem mass spectra were also recorded for each standard using several different "% collision energy" values.

TLC/DESI-MS. The DESI-MS setup with manual and computer-controlled *x*, *y*, *z* sample stage is shown in Figure 2. Analysis of the large area presented by a 10 × 10 cm TLC plate required that the atmospheric sampling heated capillary be extended out from the main body of the instrument. To accomplish this, a shallow counterbore (~1 mm) was drilled into the heated sampling capillary (550-μm i.d.). A 10.4-cm-long, 20-gauge stainless steel tube extension (597-μm i.d., 902-μm o.d., Scientific Instrument Services, Inc. Ringoes, NJ) was press-fit into the counterbore. The thermal expansion of the capillaries under normal operation of the source and the press-fit sealed the extension in place. A slight bend in the capillary toward the surface was made at the atmospheric sampling end to keep the capillary above the surface being analyzed except at the point of analysis.

The DESI emitter used for these experiments was mounted from the front flange of the mass spectrometer. The spray emitter was a 5.2-cm-long, taper-tip fused-silica capillary (50-μm i.d., 360-μm o.d., New Objective, Woburn, MA). The inner diameter of the nebulizing gas tube was 500 μm, which provided a nebulizing gas (nitrogen) jet annulus area of ~0.95×10^{-7} m^2. The ES emitter was mounted ~3.5 mm from the surface to be analyzed at an approximate 60° angle to the surface and 4 mm back from the sampling capillary. The surface was positioned so that the bottom of the sampling capillary was 50 μm or less above the surface. The nebulizer gas flow rate was set to ~2.5 L/min (70 in the instrument software) for the various experiments (~440 m/s nebulizing gas jet linear velocity). The ES high voltage was applied to the stainless steel body of the micro ion spray head. The ES solvent was delivered to the emitter by a syringe pump using a 2.5-mL glass syringe. A grounded union was placed in the transfer line (~40 cm of 127-μm i.d. ($^1/_{16}$-in. o.d.) PEEK tubing) between the syringe pump and the DESI emitter. (*Safety consideration: The DESI emitter floats at the high ES voltage and appropriate shields and interlocks should be used to avoid accidental contact with this component.*)

The MS2000 *x*, *y*, *z* robotic platform (Applied Scientific Instrumentation Inc., Eugene, OR) and the basic control software used to manipulate the TLC plate relative to the stationary DESI emitter have been described previously.[5,6,22] Glass-backed 10 cm × 10 cm TLC plates were secured with plastic lock nuts into a recess in a rectangular plexiglass block attached to the top of the positioning stage. Aluminum-backed plates were secured with double-sided tape to a 10 cm × 10 cm glass plate mounted into this recess. For initial positioning and mass spectral detection optimization, the surface position was controlled with the joystick (*x*, *y*) and jog wheel (*z*-surface-to-DESI emitter axis) of the manual control unit of the *x*, *y*, *z* sample platform. To accomplish the positioning of the sampling capillary above the surface, a black and white CCD camera and associated monitor (Protana A/S, Odense, Denmark) were used to observe the DESI emitter and sampling capillary from an angle just above the surface. A color webcam (Creative Technology, Ltd., Milpitas, CA) was mounted directly over the surface to be analyzed and centered on the DESI emitter and sampling capillary. The webcam image was output to the screen of the surface control software running on the mass spectrometer PC. This "bird's eye" view of the surface was used for manual positioning and computer-controlled scanning of the surface in the *x*,*y* plane. The surface could be illuminated with white or long-wave UV light.

RESULTS AND DISCUSSION

Optimization of the ES-MS and TLC/DESI-MS Conditions. Five alkaloid standards were investigated with conventional ES-MS by spraying methanol solutions of the individual standards. The fixed-charge analytes berberine ($(\mathbf{4})^+$) and palmatine ($(\mathbf{7})^+$) were detected at *m/z* 336 and 352, respectively. Hydrastine (**10**) and tetrahydroberberine (**6**) were detected as protonated molecules at *m/z* 384 and 340, respectively. No signal corresponding to the protonated molecule of hydrastinine (**1**) was observed at the expected *m/z* 208. Rather, an intense peak was observed at *m/z* 190, consistent with the elimination of water from protonated hydrastinine, i.e., $(\mathbf{1} + H - H_2O)^+$ or $(\mathbf{1a})^+$. This same ion was observed as the base peak when spraying an acetonitrile solution of hydrastinine. We did not independently confirm the structure of the standard material used to prepare our solutions of hydrastinine. However, literature reports of the GC/MS and ES-MS analysis of hydrastinine, in which the structure of the standard material was checked, also note *m/z* 190 as the base peak with

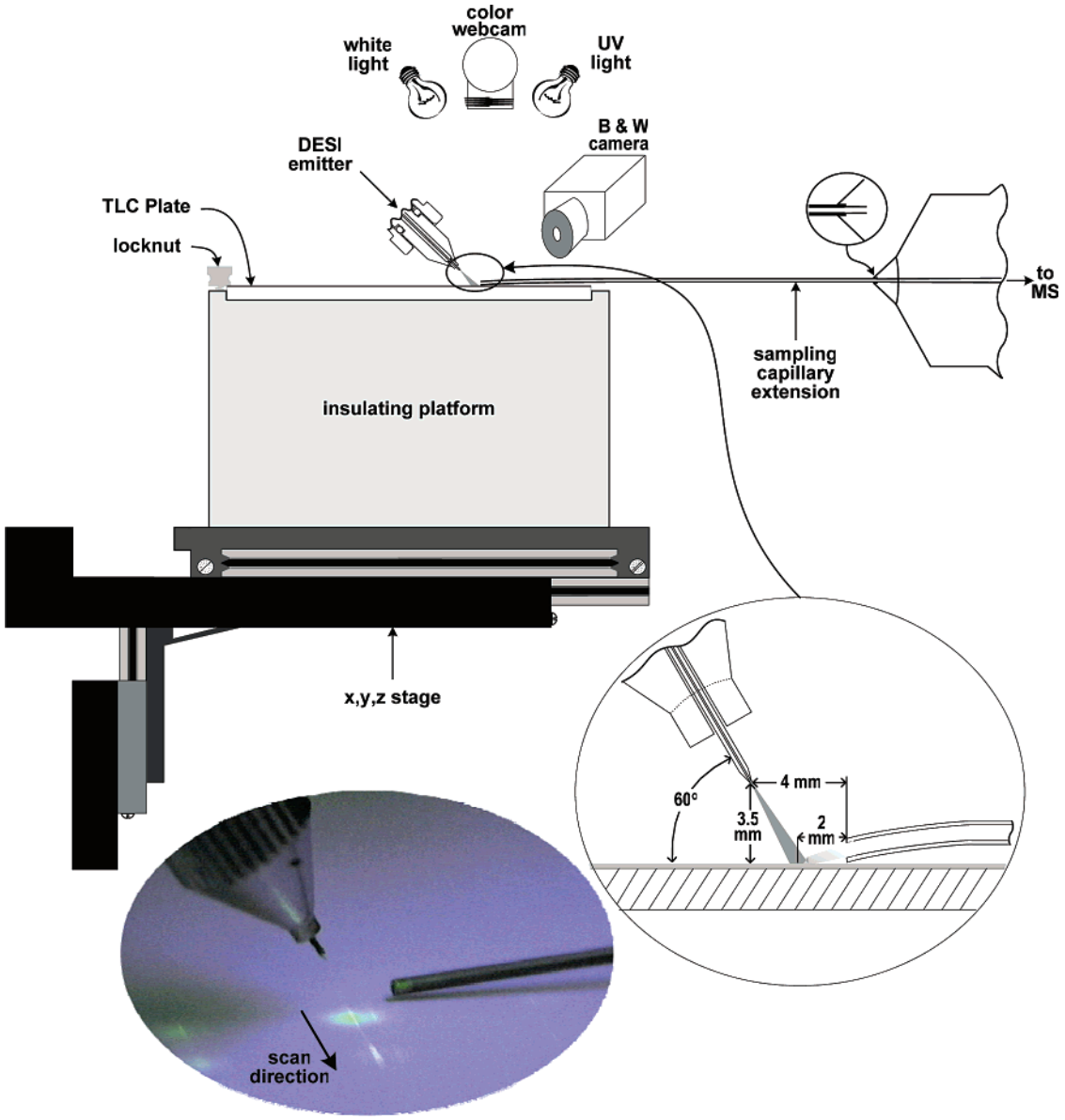

Figure 2. Schematic side view illustration of the DESI-MS setup. Line drawing inset shows the optimized DESI emitter positioning relative to the TLC plate surface and sampling capillary extension. Color photograph inset shows the same region during an actual surface scan along a development lane.

little or no ion intensity at m/z 208.[27] Apparently, hydrastinine is easily dehydrated under typical ES-MS conditions to form a species with quaternary ammonium structure $(\mathbf{1a})^+$, or similar structure.

For TLC/DESI-MS detection optimization, a standard mixture of five alkaloids (5 nmol of each, hydrastinine (**1**), hydrastine (**10**), palmatine ($(\mathbf{7})^+$), berberine ($(\mathbf{4})^+$), and tetrahydroberberine (**6**)) was spotted in multiple lanes on a normal-phase aluminum-backed TLC plate and the plate was developed to separate the compounds (e.g., Figures 3a and b). The TLC separation achieved was consistent with those reported in the literature.[25] Hydrastinine did not move far from the origin and exhibited a characteristic light blue fluorescent spot under long-wave UV conditions. Palmatine and berberine exhibited distinct yellow or yellow-green bands at higher R_f values, which were readily visible under long-wave UV or under visible light when higher amounts were spotted. The hydrastine band, which exhibited a weak blue fluorescence under long-wave UV light, overlapped with the low R_f side of the palmatine band. Tetrahydroberberine was not readily observed on the plate. Later, mass spectrometric analyses determined that it was, indeed, on the plate, but under the conditions of this development, at high R_f, as discussed below.

The angle of the DESI emitter to the surface, the distance of the emitter from the surface, and the distance of the emitter to the sampling capillary were all optimized to achieve maximum alkaloid signal in a series of surface scanning experiments. Each development lane was scanned by moving the plate relative to the stationary DESI probe at 100 μm/s from a point just below the spotting point to a position 50 mm (8.3-min surface scan time) up the lane, which was beyond the berberine band. This scan apparently ended before reaching the band for tetrahydrober-

(27) Weber, H. A.; Zart, M. K.; Hodges, A. E.; Molloy, H. M.; O'Brien, B. M.; Moody, L. A.; Clark, A. P.; Harris, R. K.; Overstreet, J. D.; Smith, C. S. *J. Agric. Food Chem.* **2003**, *51*, 7352−7358.

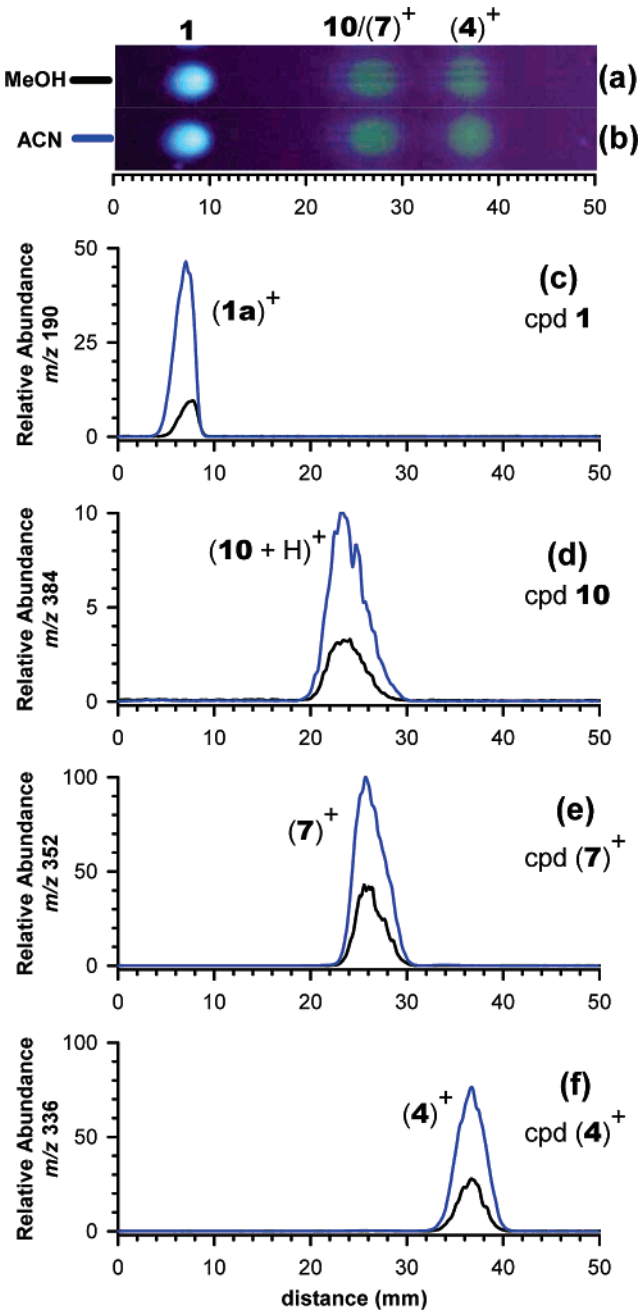

Figure 3. Postanalysis photographs of replicate 5-cm-long TLC plate development lane segments in which the aluminum-backed TLC plate was scanned relative to the DESI emitter along the development lane using (a) methanol or (b) acetonitrile at 10 μL/min. Separated components from the spotted mixture (5 nmol each component) were hydrastinine (**1**, detected as (**1a**)$^+$, *m/z* 190), hydrastine (**10** + H)$^+$, *m/z* 384), palmatine ((**7**)$^+$, *m/z* 352), and berberine ((**4**)$^+$, *m/z* 336) from low to high R_f. The black line (methanol) and blue line (acetonitrile) traces are the extracted ion current profiles for (c) *m/z* 190, (d) 384, (e) 352, and (f) 336 obtained during the lane scans. All plots were normalized to the signal levels for palmatine obtained using acetonitrile as the desorption solvent (blue line trace, in (e)). The development lanes were scanned from the spotting origin at 100 μm/s, while full-scan mass spectra were collected (*m/z* 50−500).

berine, and thus, this compound was not detected in these experiments. The plate was aligned so that the development lane was at 90° to the sampling capillary (see colored photo in Figure 2). During the surface scan along a lane, full-scan mass spectra (*m/z* 50−500) were recorded (~1 scan/s). Ions of the same *m/z* values as those observed for the respective compounds in ES-MS were observed in these TLC/DESI-MS experiments. Ultimately, the positioning illustrated in the line drawing inset in Figure 2 was determined optimum and used to acquire all subsequent data.

Four DESI "spray solvents", viz., acetonitrile and methanol, with and without 0.1% (v/v) acetic acid, were evaluated at flow rates of 5 and 10 μL/min. Acetonitrile produced ~2−5 times more signal than methanol (Figure 3c−f). The presence of acetic acid in either solvent did not enhance the signals from the alkaloids. In fact, the addition of the acid led to an increase in the background noise level as well as slightly lower signal levels from the alkaloids. The higher DESI solvent flow rate produced greater signal as a result of a larger desorption plume impacting the surface. At 10 μL/min, the DESI plume tracked a desorption trail ~0.8 mm wide. Unmodified acetonitrile at flow rate of 10 μL/min was used for the remainder of the experiments discussed below.

The "droplet pickup mechanism"[1] was apparently responsible for the sampling and ionization of the alkaloids on the TLC plate surface. The charged solvent droplets impacting the surface dissolved some of the analyte, and charged droplets containing the analyte were subsequently liberated from the surface by mechanical force of the impacting droplets or nebulizing gas. Once an analyte was present in the charged droplets leaving the plate, the normal mechanisms that give rise to gas-phase ions from analytes in the droplets in ES ionization were presumably in operation. The differences in signal levels among methanol and acetonitrile appeared to be caused by two competing processes: (a) dissolution and subsequent desorption of the analyte from the surface and (b) dissolution and elution (development) of analyte material away from the DESI plume before desorption could take place.

This elution versus desorption phenomenon is illustrated in the photos of the development lanes in Figure 3a and b. In the top lane (Figure 3a), two surface scans were made along the development lane using methanol as the solvent. Two distinct tracks were observed on the bands where some portion of the analyte in the band was eluted from the center of the DESI plume impact area to the edges of the plume. This can be seen by the light color tracks with more intense color at the edges of the tracks. This same phenomenon is visible in the color photo inset in Figure 2. In the bottom lane (Figure 3b), acetonitrile was used as the DESI solvent and these tracks are much less apparent. In this case, the material was not rapidly eluted out from the area of the plume, and thus, desorption or ionization was more efficient. This is consistent with the fact that the alkaloids are very soluble in methanol and methanol ($\epsilon^\circ = 0.95$) is a considerably stronger developing solvent on normal-phase silica plates than is acetonitrile ($\epsilon^\circ = 0.65$).[28]

Generation of Calibration Data and Calculation of the TLC/DESI-MS Detection Limits. Initial estimates for the detection limits for berberine, palmatine, hydrastine, and hydrastinine were performed by spotting an aluminum-backed silica gel plate with 5.0-μL aliquots of a standard mixture containing 1000, 100, 10, 1, 0.1, and 0.01 pmol/μL of each analyte. The plate was then

(28) Fried, B.; Sherma, J. *Thin-Layer Chromatography,* 4th ed.; Marcel Dekker: New York, 1999; p 92.

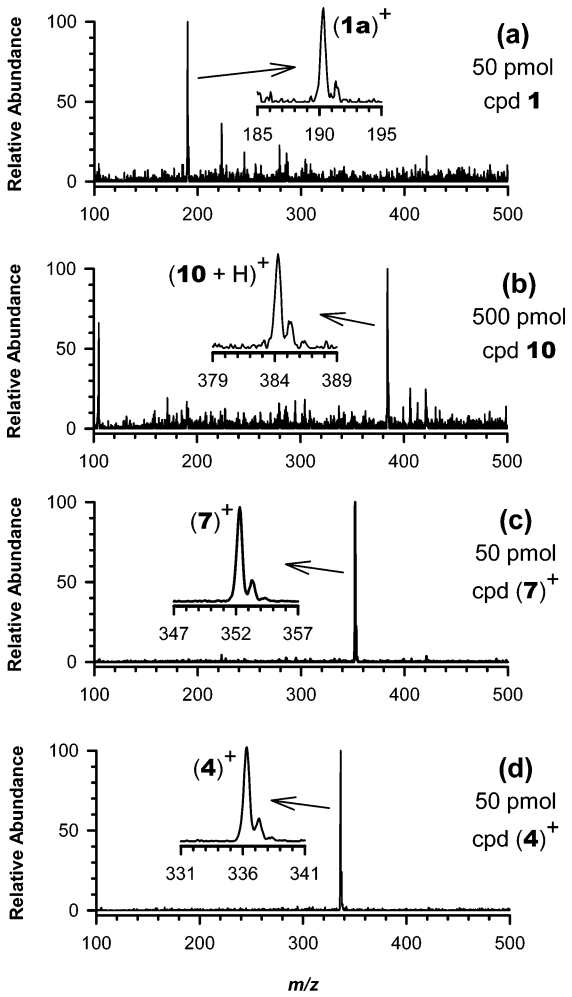

Figure 4. Full-scan mass spectra (*m*/*z* 50−500) obtained for (a) **1**, (b) **10**, (c) $\mathbf{7}^+$, and (d) $\mathbf{4}^+$, spotted as a mixture and separated on aluminum-backed TLC plates. Spectra were obtained while scanning at 100 μm/s across the respective band at constant R_f using acetonitrile spray solvent at a flow rate of 10 μL/min.

developed, and the bands corresponding to each compound were evaluated using the optimized TLC/DESI-MS conditions. For this and all quantitative work, surface scans were made across the plate at a constant R_f value in manner such that the DESI plume tracked through the center of each band. The resulting data suggested that the detection limits for berberine and palmatine were both ~5 pmol, while that for hydrastinine was ~50 pmol. Hydrastine, which was incompletely resolved from palmatine, fluoresced (blue) only weakly under long-wave UV light, making location of the center of the band less precise than for the other bands. Tetrahydroberberine could not be seen on the plates. For that reason, subsequent, more accurate, quantitative measurements focused on only three of the five alkaloid standards (see below).

The spectra in Figure 4 illustrate the quality of the mass spectra achieved for hydrastinine, hydrastine, palmatine, and berberine, near the lower levels of detection. At the levels chosen, the molecular ionic species for each of the respective compounds was the base peak in the spectrum. With the same amount of material on the plate (i.e., 50 pmol), the signal-to-background levels for berberine (Figure 4d) and palmatine (Figure 4c) were comparable and superior to that for hydrastinine (Figure 4a). Furthermore,

Table 2. Figures of Merit for the Calibration Curves and Calculation of the Detection Limit for TLC/DESI-MS

compound	berberine	palmatine	hydrastinine
calibration range, pmol	2.5−100	2.5−100	5−100[a]
slope (b) ± std dev	2.6 ± 0.11	3.9 ± 0.16	0.52 ± 0.044
intercept (a) ± std dev	9.1 ± 5.3	3.2 ± 7.4	−1.5 ± 2.2
r^2	0.98	0.98	0.95
standard error of the y value estimates, $s_{x/y}$ or sB	14	19	4.8
detection limit, pmol ($3sB/b$)	16	14	28
molecular weight, pg/pmol	336	352	207
detection limit, ng	5.3	5.1	5.8

[a] TLC plates were spotted with 2.5 pmol of hydrastinine, but no signal was observed.

~10 times more hydrastine (500 pmol, Figure 4b) was required to achieve a mass spectrum with quality comparable to 50 pmol of hydrastinine (Figure 4a) Neither hydrastine nor hydrastinine is a fixed-charge ion, but rather must be protonated to be detected. Hydrastinine was detected at *m*/*z* 190 rather than the expected *m*/*z* 208 (nominally $(\mathbf{1} + H - H_20)^+$), indicating that it was dehydrated somewhere in the analysis process to produce a species that carried a fixed charge (conversion of **1** to $(\mathbf{1a})^+$, Figure 1). This might relate to its superior performance compared to that of hydrastine. The behavior of hydrastine might be related to the fragility of the molecule compared to the others studied, but no evidence of fragmentation was evident in the mass spectra. The poorer signal levels achieved for hydrastine were not simply related to interaction with the stationary phase either, because this compound was located on the plate at near the same R_f level as palmatine. Indications are that the fixed-charge analytes studied were desorbed/ionized or sampled more efficiently in the TLC/DESI-MS experiment than those analytes that required protonation. More study is warranted to determine the physical or chemical mechanisms responsible for these observations.

Two calibration curves employing different ranges were used to determine the detection limits for hydrastinine, palmatine, and berberine more exactly. Aluminum-backed plates were spotted with duplicate 1.0-μL aliquots containing (a) 1000, 500, 250, 100, 50, and 25 pmol/μL and (b) 100, 50, 25, 10, 5, and 2.5 pmol/μL of the standard mixture prepared in methanol. The plates were developed and the individual spots scanned with the TLC/DESI-MS system. The integrated peak areas from the extracted ion current profiles for the respective molecular ionic species were determined.

Linear calibration data were obtained for all three target analytes using both calibration curves, but the lower range data were used for calculating detection limits. The calibration data were evaluated using a least-squares regression and fit the model $A = bx + a$, where A is the integrated peak area for a compound with mass x spotted on the TLC plate. The values b and a are the slope and intercept, respectively, of the calibration curve and are presented for each compound in Table 2. In all cases, the coefficient of determination, $r^2 > 0.94$, was considered reasonable because all compounds were being detected directly on a TLC plate without the benefit of an internal standard from full-scan mass spectra. Calibration curves employing higher order fits were also evaluated; however, adding additional terms to the fit did not explain significantly greater variability. From the linear calibration

Table 3. Figures of Merit for the Calibration Curves and Calculation of the Detection Limit for TLC/Fluorescence

compound	berberine	palmatine	hydrastinine
calibration range, pmol	2.5−100	2.5−100	2.5−100
slope (b) ± std dev	320 ± 71	360 ± 44	330 ± 58
intercept (a) ± std dev	480 ± 3500	1100 ± 2100	1700 ± 2700
r^2	0.96	0.98	0.97
standard error of the y value estimates, $s_{x/y}$ or sB	3800	2400	3100
detection limit, pmol ($3sB/b$)	36	20	28
molecular weight, pg/pmol	336	352	207
detection limit, ng	12	7.0	6.0

curves, the detection limit was estimated ($3\ s_{x/y}$/slope, where $s_{x/y}$, the standard error of the y value estimates, is assumed to approximate the standard deviation of the blank, s_B.)[29] For each of the three analytes, the detection limit was approximately 5−6 ng/analyte, as shown in Table 2. This translates to 16, 14, and 28 pmol for berberine, palmatine, and hydrastinine, respectively.

While these statistically determined detection levels were not exceptionally low for ES-MS, these values were comparable with the 1−100 ng (0.1−10 ng for HPTLC) detection limit range typical for fluorescence-based detection on TLC plates.[23] To examine this further, a set of calibration standards identical in concentration and mass used for the TLC/DESI-MS analyses were evaluated with fluorescence detection. Linear calibration curves were calculated, with the figures of merit and detection limits presented in Table 3. In general, $r^2 > 0.96$ was found for all three analytes. Again, the addition of higher order (e.g., quadratic) terms to the calibration curves did not explain additional variability. The calculated detection limits of 6−12 ng, which translates to 36, 20, and 28 pmol for berberine, palmatine, and hydrastinine, respectively, were comparable to those detection levels determined by DESI-MS.

TLC/DESI-MS detection levels would be enhanced further with newer more sensitive instrumentation or possibly with more selective detection on instruments such as a triple quadrupole. However, improved chromatography would also improve detection limits. With the manual spotting and the development methods used here, analyte bands ranged in diameter from ~3 (low R_f) to as much as 5 (high R_f) mm. Given that the DESI plume tracking through the bands was only 0.8 mm wide, a large fraction of the total material in a band was left on the plate rather than sampled into the mass spectrometer. The area of the bands can be estimated by the area of a circle (πr^2, where r = radius), while the area of the bands sampled can be estimated as $2rw$, where w represents the width of the DESI plume track at the center of the band (i.e., 0.8 mm). The percentage of the material uniformly distributed in a band that could possibly be sampled by the DESI plume (SA%) is thus expressed as the ratio of these two areas (eq 1).

$$\mathrm{SA\%} = (2w/\pi r) \times 100 \qquad (1)$$

In the case of 3- and 5-mm-diameter TLC bands, the percentage of the band area sampled was ~34 and ~20%, respectively. Thus, TLC/DESI-MS detection levels would be enhanced significantly by a reduction in the diameter of the developed bands. Optimally this diameter should be less than the width of the DESI plume so 100% of the band area can be probed. Alternatively, the width of the DESI plume could be increased beyond that used here to desorb more of a band from the plate. It is not clear, however, if the larger desorption region would be efficiently sampled into the mass spectrometer, resulting in a linear increase in signal with increasing plume size. In any case, increasing the plume diameter significantly would sacrifice lateral readout resolution in surface scans along a development lane.

TLC/DESI-MS and Fluorescence Quantification of Goldenseal Alkaloids in Two Commercial Dietary Supplements. TLC/DESI-MS was used to quantitate berberine, palmatine, and hydrastinine in two of the commercially available goldenseal brands, viz., Solgar and Nature's Resource. Among those brands tested, both of these had a laboratory-based assay for berberine or total alkaloids listed on the product label, making it possible to check our analytical results with label claims. Qualitative analysis with TLC/DESI-MS (see below) found that the Solgar sample was a complex mixture containing berberine and palmatine as major constituents, among other alkaloids. The Nature's Resource sample, by contrast, contained berberine as its major constituent with much lesser amounts of other alkaloids. Both brands contained hydrastinine as a minor constituent. The integrated peak areas from quadruplicate TLC/DESI-MS analyses were compared against those from the high-level linear calibration curve (25−1000 pmol of each analyte) and used to calculate the mass of hydrastinine, palmatine, and berberine in a typical capsule.

For both brands there was reasonable agreement between the TLC/DESI-MS results calculated and the values noted in the label value (Table 4). For example, each Solgar capsule was expected to contain 15 mg of total alkaloid; the TLC/DESI-MS result was ~18 mg, based on the sum of the three species we measured. Each Nature's Resource capsule was expected to contain 13.4 mg of berberine; the TLC/DESI-MS result was ~12 mg. The relative standard deviation (RSD) for the values in question was ~15% in the case of the Solgar sample and ~8% for the Nature's Resource sample. Smaller RSD values might be expected with the use of an internal standard.

Fluorescence quantification of alkaloids in the Solgar and Nature's Resource goldenseal brands was also performed (Table 4). The mass of berberine calculated in the Natures's Resource brand using fluorescence analysis (14 mg/capsule) agreed well with that calculated using TLC/DESI-MS (12 mg/capsule) and the label value (13.4 mg/capsule). The mass of berberine calculated in the Solgar goldenseal using fluorescence (19 mg/capsule) was high relative to both the values from TLC/DESI-MS (16 mg/capsule) and the label value (15 mg of total alkaloid/capsule). Furthermore, the masses of palmatine calculated in the Solgar goldenseal using fluorescence and TLC/DESI-MS were quite different, viz., 8.4 and 2.2 mg/capsule, respectively. On the basis of the more extensive qualitative analysis, we believe that additional species at the same R_f value on the plate interfered with the fluorescence measurements, resulting in a larger value than that obtained using TLC/DESI-MS. The TLC/DESI-MS-derived ion map discussed in the section below definitively showed the

(29) Miller, J. C.; Miller, J. N. *Statistics for Analytical Chemistry,* 2nd ed.; Ellis Horwood Ltd: Chichester; 1988; pp 110−115.

Table 4. Quantification Results for Goldenseal Alkaloids in Two Commercially Available Brands Determined Using TLC/DESI-MS and Fluorescence Spectroscopy, and Compared with Label Values

	method of quantitation	calculated mass of alkaloid per capsule, mg[a]		
		berberine	palmatine	hydrastinine[b]
Solgar	TLC/DESI-MS	16 ± 2.3; $n = 4$	2.2 ± 0.37; $n = 4$	<0.24
	fluorescence	19 ± 0.86; $n = 3$	8.4 ± 0.47; $n = 3$	<0.24
	label value[c]	15		
Nature's Resource	TLC/DESI-MS	12 ± 0.91; $n = 4$	not detected	<0.24
	fluorescence	14 ± 1.2; $n = 3$	not detected	<0.24
	label value[d]	13.4		

[a] Reporting convention is mean ± standard deviation based on n replicates. [b] Hydrastinine was observed, but at a mass below its calculated detection limit of 0.24 mg/capsule for both DESI-MS and fluorescence. [c] Estimated total alkaloid content is 15 mg/capsule, based on the label values. This value has been assigned to berberine for comparison purposes. [d] Label values also includes 10.7 mg of hydrastine/capsule.

multiple overlapping species in this region of the TLC plate for the Solgar sample. The selectivity of the mass spectrometric detection alleviated the problem of quantification in the case of overlapping components.

Qualitative Identification of the Goldenseal Alkaloids in Commercial Dietary Supplements. The TLC separations of the extracts from the six commercially available goldenseal capsules or tablets examined showed that there was considerable variation among the samples in terms of the actual alkaloids present and in the amounts of these different compounds (Table 1 and Figure 5). The major alkaloids were identified initially by comparing the R_f values of the bands from the extracts to those of the alkaloid standards separated in a lane on the same TLC plate. These identifications were confirmed and more identifications were made, some tentative, using TLC/DESI-MS (Table 5).

The TLC/DESI-MS results confirmed that all samples contained significant quantities of berberine, the major alkaloid expected from an authentic goldenseal sample, and a number of botanical adulterants sold as or admixed with goldenseal. The NOW and GNC samples contained berberine almost exclusively with only a trace of hydrastinine detected in the former and a trace of hydrastine in the latter. Hydrastinine is a degradation product of hydrastine, and therefore, the NOW sample must have contained at one point a small amount of hydrastine. Hydrastine reportedly is specific to goldenseal.

The Nature's Resource and Solaray samples appeared to be similar by inspection of the optical image of the TLC plate. The mass spectral data confirmed that these two samples were similar in composition, but they did contain, in addition to berberine, detectable amounts of other alkaloids including hydrastine, and species at m/z 352 and 370, tentatively identified as berberastine (**8**) and canadaline (**9**), respectively. These proposed identifications were based on the observed m/z and the known alkaloids in goldenseal.[25]

The Walgreen's goldenseal root powder and Solgar goldenseal/goldthread root complex extract were similar in composition and more complicated mixtures than the other samples examined. The optical image of the plate showed that these two samples had one band different from all the other samples examined at an R_f value between hydrastinine and hydrastine/palmatine. The mass spectral data revealed a rich variety of compounds on the TLC plate for both samples, including significant quantities of berberine, palmatine, and at least three other compounds, which were observed at m/z 336 (the band unique to these two samples), 320 and 338, from lower to higher R_f, respectively. We believe these ions are indicative of epiberberine ($(\mathbf{3})^+$), coptisine ($(\mathbf{2})^+$), and jatrorrhizine ($(\mathbf{5})^+$), respectively. Berberine occurs in goldenseal and berberine, epiberberine, palmatine, coptisine, and jatrorrhizine are known components of the particular goldthread species (*C. chinensis*)[30] named on the Solgar product label. In additional experiments (data not shown), we found the product ion spectrum of the species at m/z 336 in the unique band was consistent with that reported for epiberberine in the literature and different from the product ion spectrum of berberine.[30,31] Similarly, the product ion spectrum of the species at m/z 320 was consistent with that reported for coptisine[30,31] with the base peak in our spectrum at m/z 292. Also, the location on the plate for the species identified as jatrorrhizine in the samples was at the same R_f value for an authentic standard of this material. This supports the identification of this component as jatrorrhizine. Further differentiation from the isomeric columbamine was not possible because a standard was not available for comparison and because the product ion spectra of the two are very similar.[30,31] The comparable composition of the Walgreen's goldenseal sample to the Solgar sample strongly suggested the presence of at least one additional and undeclared herb in that product, possibly goldthread.

A particularly significant finding was the positive identification of the location of the epiberberine and jatrorrhizine on the plate relative to the other alkaloids. A major compendium that presents the TLC separation of goldenseal and *Coptis spp.* under conditions identical to those used here assigned the epiberberine band as jatrorrhizine in a *Coptis spp.* sample.[25] Our mass spectral data show that jatrorrhizine actually appears at an R_f value intermediate to that of palmatine and berberine rather than at an R_f value below hydrastine. Also consistent with this misidentification was the observation in the compendium and in our work of the strong fluorescence of the band we believe to be epiberberine. The fluorescence intensity of jatrorrhizine was much less that that observed for equal moles of palmatine or berberine.

The summed extracted ion current profiles from the scans along the development lanes of the TLC plate as shown in Figure 5 did not adequately display the rich data set provided by the full-scan mass spectra. This was particularly true in the case of

(30) Wu, W.; Song, F.; Yan, C.; Liu, Z.; Liu, S. *J. Pharm. Biomed. Anal.* **2005**, *37*, 437−446.
(31) Chang, W.-C.; Young, D.-S.; Kao Liu, L.; Sheu, S.-J. *J. Chromatogr., A* **1996**, *755*, 19−26.

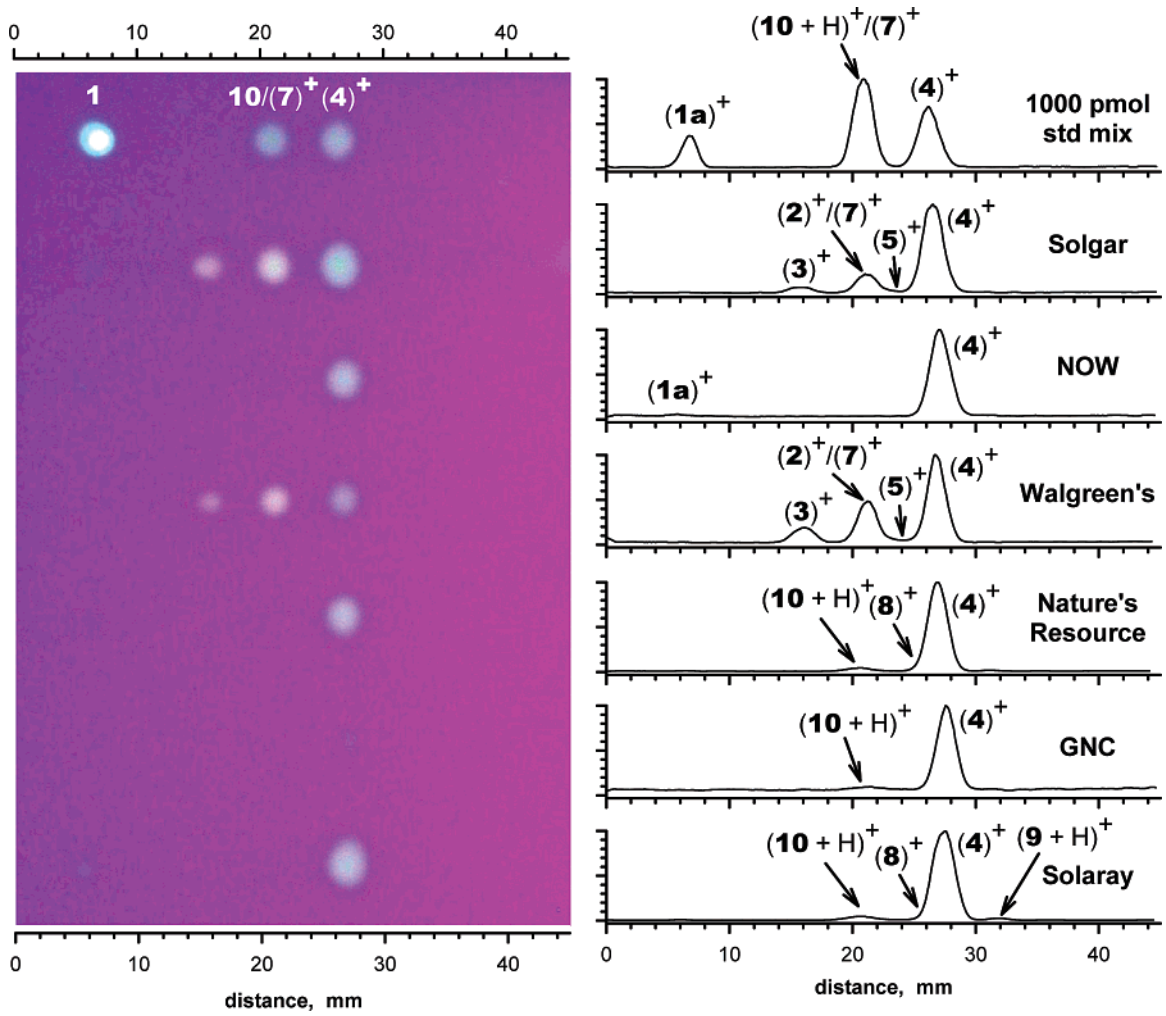

Figure 5. Photograph of normal-phase TLC separations of a mixture of **1**, **10**, $\mathbf{7}^+$, and $\mathbf{4}^+$ standards (1000 pmol each) and six different goldenseal supplements on a single glass-based plate. The corresponding summed extracted ion current profiles for all the alkaloid species observed during the different analyses (summed ion current for *m*/*z* 190, 320, 336, 338, 340, 352, 370, and 384). Intensities in each development lane are normalized to the highest ion current recorded in that respective development lane. A 45-mm section of each development lane beginning at the spotting origin was scanned at 100 μm/s, while full-scan mass spectra were collected (*m*/*z* 50−500). DESI spray solvent was acetonitrile at 10 μL/min.

the Solgar and Walgreen's samples. An ion map, such as that in Figure 6 for the Solgar sample, bettered showed the complexity of the mixture and made more noticeable the minor alkaloids in the sample. At the appropriate upper and lower intensity display limits, one could see easily, from low to high R_f, the five major alkaloids mentioned above, viz., epiberberine, hydrastine, palmatine, coptisine, jatrorrzhine, and berberine. Note that coptisine and palmatine were completely overlapped and were not apparent by simple visual inspection of the plate. Hydrastine also was largely overlapped with this same major palmatine band. Jatrorrhizine was not observed visually on the plate even though it was between the two major bands. This was apparently because of its low concentration and the fact that it does not have the same magnitude of fluorescence as the other compounds. Also observed in this view at higher R_f were what we tentatively identified as canadaline and tetrahydroberberine. The identification of tetrahydroberberine was supported by other experiments that found the compound at *m*/*z* 338 in the Solgar sample and the standard compound had the same R_f value. Other trace species that might be alkaloids, such as a second species at *m*/*z* 352 (possible berberastine) and a species at *m*/*z* 368 (unknown), among others, were also visible in the ion map.

CONCLUSIONS

DESI-MS was shown here to provide the capability to sample, ionize, identify, and quantify goldenseal and related alkaloids directly from developed normal-phase (glass- or aluminum-backed) TLC plates. The ability to identify and quantitate species directly from the surface of an intact TLC plate as large as 10 × 10 cm, at atmospheric pressure, without additional postdevelopment processing, and without operator intervention, represents a significant advantage over other TLC/MS methodologies reported in the literature. In theory, with the appropriate extension of the mass spectrometer sampling capillary and the proper surface position system, this approach could be extended to surfaces of any given size, including the common 10 × 20 cm or 20 × 20 cm TLC plates.

Standard curves for the fixed-charge alkaloids berberine, palmatine, and hydrastinine (detected as $(\mathbf{1a})^+$) were linear over two separate ranges, viz., 2.5−100 and 25−1000 pmol. The detection levels for these three alkaloids, obtained in mass spectral

Table 5. Compounds Detected in Goldenseal Supplements Corresponding to Analysis of TLC Plate Shown in Figure 5

				standards and samples[a]					
cpd	*m/z*	rel R_f[b]	1000 pmol std mix[c]	Solgar	NOW	Walgreens	Nature's Resource	GNC	Solaray
hydrastinine, detected as $(\mathbf{1a})^+$	190	0.14	++	+	+	−	+	−	+
epiberberine, $(\mathbf{3})^+$	336	0.37	−	++	−	++	−	−	−
hydrastine, $(\mathbf{10} + H)^+$	384	0.47	++	+	−	+	++	+	++
coptisine, $(\mathbf{2})^+$	320	0.48	−	++	−	++	−	−	−
palmatine, $(\mathbf{7})^+$	352	0.50	++	++	−	++	−	−	−
jatrorrhizine, $(\mathbf{5})^+$	338	0.56	−	+	−	+	−	−	−
berberastine, $(\mathbf{8})^+$	352	0.57	−	+	−	+	++	−	++
berberine, $(\mathbf{4})^+$	336	0.62	++	++	++	++	++	++	++
canadaline, $(\mathbf{9} + H)^+$	370	0.71	−	−	−	−	++	−	++
tetrahydroberberine,[4] $(\mathbf{6} + H)^+$	340	0.81	+	−	−	−	−	−	−

[a] ++, detected; +, possible trace; −, not detected [b] R_f value calculated on basis of spotting origin (left edge of photo in Figure 5) to end of 45-mm section of plate shown, not to the actual solvent front which was farther to the right of the plate region shown. [c] 1000 pmol each of hydrastine, hydrastinine, palmatine, berberine, and tetrahydroberberine. [d] Detected in the standard mixture at only trace levels with the separation and DESI-MS parameters used. This compound was better detected in the standard mix and was detected at trace levels in the Solgar sample when each was investigated using shorter development times (distances) (see Figure 6).

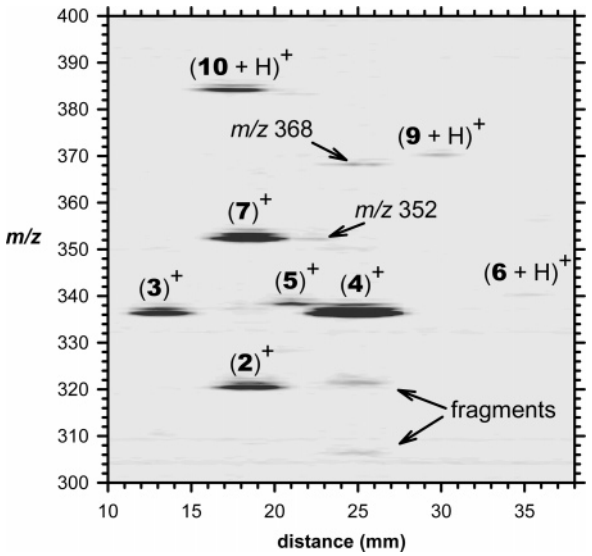

Figure 6. Ion map derived from the TLC separation of the Solgar sample on an aluminum−backed plate and subsequent TLC/DESI-MS analysis of the development lane. The development lane beginning at the spotting origin was scanned at 100 μm/s, while full-scan mass spectra were collected (*m/z* 50−500). The region from 10 to 38 mm and *m/z* 300−400 is shown to accentuate the overlapping and minor species in this region of the plate. Development time was shorter for this plate than for the other plates shown in this report resulting in shorter development distances for all compounds on the plate. DESI spray solvent was acetonitrile at 10 μL/min.

full-scan mode, were ∼5 ng (∼14−28 pmol) for each alkaloid. However, signal levels for hydrastine, which required protonation for detection, were nearly 1 order of magnitude lower than that for a comparable amount of the fixed-charge alkaloids. We also had difficulty at times detecting tetrahydroberberine. All signal levels for a given amount of alkaloid would be improved on the same instrumentation by superior chromatography that resulted in smaller analyte bands. With smaller bands, a larger portion of the total material would be sampled by the DESI spray plume. Detection limits might also be improved by the use of more advanced mass analyzer systems and specific detection modes like selected reaction monitoring. Nonetheless, these levels of detection are comparable to fluorescence detection and should be adequate for typical sample loadings on conventional TLC and HPTLC plates.

Qualitative screening of the major alkaloids present in six different over-the-counter goldenseal dietary supplements showed that the samples varied in terms of the actual alkaloids present and in the amounts of these different compounds. Mass measurements and product ion spectra were used to correct a literature misassignment of the epiberberine band as jatrorrhizine in goldthread.[25] In one sample, alkaloids were detected that strongly suggested the presence of at least one additional herb undeclared on the product label. Quantities of the alkaloids present in two of the samples determined using the mass spectral data were in reasonable agreement with the label values indicating the quantitative ability of the method. An advantage afforded by the mass-specific detection for quantitation over fluorescence-based quantitation was illustrated in the case of overlapping analyte bands on the plate.

In summary, the results presented here further demonstrate the potential of DESI as a general interface for TLC plate readout with mass spectrometric detection. Questions related to DESI-MS detection capabilities for other than fixed-charge ions will need to be studied further to assess the range of compounds applicable to detection and at what levels by this approach to TLC/MS. The related desorption atmospheric pressure chemical ionization might provide a means to analyze more nonpolar molecules separated on normal-phase and other separation phases that are less applicable to ionization by electrospray mechanisms.

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