Fast screening of low-MW compounds is performed by thin-layer chromatography (TLC) followed by direct on-spot matrix-assisted laser desorption/ionization time-of-flight mass spectrometry identification with nearly “matrix-free” mass spectra using an UV-absorbing ionic liquid matrix. Owing to minimal background ions from the proton donor triethylamine/α-cyano-4-hydroxycinnamic acid ionic liquid matrix, three arborescidine alkaloids, the anesthetics levobupivacaine and mepivacaine, and the antibiotic tetracycline were readily characterized most frequently by the MS detection of their protonated molecular ions. The technique is fast and sensitive, requires little sample preparation and manipulation, and is therefore suitable for fast screening with TLC separation and MS identification of low-MW compounds, with potential applications in areas such as phytochemistry, synthetic chemistry, and product manufacturing quality monitoring.

The broad success of matrix-assisted laser desorption/ionization (MALDI)¹ has been mainly related to the ability of the matrix to incorporate and transfer the laser radiation energy to high molecular weight (MW) molecules such as polymers, sugars, and proteins.² In MALDI, the analyte is dissolved into a solid ultraviolet-absorbing organic acid matrix, which vaporizes upon laser radiation carrying with it the analyte.³ Owing to chemical noise from matrix ions in the low-m/z range, MALDI is however not so widely applicable for low-MW compounds. Direct desorption/ionization without a matrix has been applied, but its use is restricted owing to the rapid analyte degradation often observed upon direct exposure to laser radiation.⁴

The analytical technique described herein for low-MW compound screening applies thin-layer chromatography (TLC) and direct “on-spot” positive ion MALDI time-of-flight (TOF) mass spectrometry (TMT-MS) using an UV-absorbing proton donor ionic liquid as the matrix. TLC is a simple and fast separation technique mainly used for “light” organic molecules. “On the bench” TLC experiments analyze many samples simultaneously at low cost and with minimal equipment and operator training. Identification in TLC is made by comparison of retention times (Rf) against those of standards, but if an unknown or unexpected spot appears, its identity has to be verified by other more refined and time-consuming techniques such as GC/MS or HPLC/MS. The possibility to perform on-spot mass analysis in TLC is therefore very attractive.

To accomplish on-spot mass analysis, TLC has been coupled with fast-atom bombardment, liquid secondary ion, laser desorption, and electrospray ionization (ESI) mass spectrometry.⁵ The combination of TLC with MALDI-TOF MS offers, however, the advantage of very high sensitivity and speed with minimal analyte spreading and spot manipulation. TLC with on-spot MALDI-TOF MS has already been tested, therefore, for polymers, peptides and proteins,⁶ nucleotides,⁷ and styrene oligomers.⁸ Dyes,⁹ drugs,¹⁰ and pesticides¹⁰ have also been analyzed, but for those low-MW analytes, the background ions from the matrix often causes serious interferences. Another key to successful TMT-MS for on-spot analysis is the method for matrix application.²¹

In phytochemistry, organic synthesis, and manufacturing process quality monitoring, low-MW analytes are often the targets; hence, the use of MALDI matrices that produce minimal noise would greatly improve TMT-MS in such areas. Herein we report that, by applying a convenient UV-absorbing ionic liquid directly on TLC spots, matrix noise is minimized (nearly eliminated) and on-spot TMT-MS analysis of low-MW compounds is conveniently performed.

Fast Screening of Low Molecular Weight Compounds by Thin-Layer Chromatography and “On-Spot” MALDI-TOF Mass Spectrometry

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Fast screening of low-MW compounds is performed by thin-layer chromatography (TLC) followed by direct on-spot matrix-assisted laser desorption/ionization time-of-flight mass spectrometry identification with nearly “matrix-free” mass spectra using an UV-absorbing ionic liquid matrix. Owing to minimal background ions from the proton donor triethylamine/α-cyano-4-hydroxycinnamic acid ionic liquid matrix, three arborescidine alkaloids, the anesthetics levobupivacaine and mepivacaine, and the antibiotic tetracycline were readily characterized most frequently by the MS detection of their protonated molecular ions. The technique is fast and sensitive, requires little sample preparation and manipulation, and is therefore suitable for fast screening with TLC separation and MS identification of low-MW compounds, with potential applications in areas such as phytochemistry, synthetic chemistry, and product manufacturing quality monitoring.

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(5) For a revision, see: Wilson, I. D. J. Chromatogr., A 1999, 856, 429.
EXPERIMENTAL SECTION

Commercial TLC aluminum sheets were used (0.25-mm silica gel with fluorescent indicator, Alugram SIL G/UV 254, Macherey-Nagel). The α-ciano-4-hydroxycinnamic acid ionic liquid matrix (Et₃N-α-CHCA) was prepared by adding triethylamine to a solution of α-CHCA in acetonitrile, with subsequent reflux for 1 h, and solvent removal under vacuum. MALDI mass spectra were acquired on a M ircomass MALDI-TOF instrument in the reflectron mode. The main settings were as follows: pulse voltage, 2450 V; delay extraction, 100 ns; accelerating voltage, 15 kV; reflectron voltage, 2 kV. Spectra were generated by summing up 10 single spectra on the m/z 50–1000 range by shooting the laser at random positions on the target spot. Placing the TLC plate over the MALDI sample plate alters m/z calibration; hence the Et₃NH⁺ ion of m/z 101 was used for internal mass calibration.

RESULTS AND DISCUSSION

Ionic liquids are easily prepared and have many beneficial properties for MALDI, including the broad capability to dissolve organic, inorganic, and polymeric substances, good thermal stability, and low vapor pressures, and a recent study by Gross and co-workers has demonstrated the suitability of ionic liquids as MALDI matrixes. To test an ionic liquid as a suitable matrix for TMT-M S, we first collected a mass spectrum on a blank TLC spot dopped with 1 μL of the Et₃N-α-CHCA matrix (Figure 1). Nearly, just a single ion from the matrix was detected by positive ion MALDI-MS: Et₃NH⁺. As Figure 2 shows as an example for arborescidine C, the on-spot TMT-M S spectrum shows ions of great abundance arising from analyte ionization. The ions of m/z 333, 335, and 337 are easily rationalized by formation, in a dual mode of ionization, of superimposing (m/z 335) isotopologue pairs of both the molecular ion and the protonated molecule of arborescidine C, a Br-containing analyte, whereas fragment ions arising from (H)Br loss are also clearly detected. Note that the abundance of the analyte ions is greater than those from the matrix, mainly that of m/z 101, that nearly no matrix ions are detected. On-spot TMT-M S analysis of the other two alkaloids A and B afforded similar mass spectra (not shown), with abundant M⁺ and M⁺ ions and nearly undetectable matrix background ions.

(14) Different concentrations ranging from 0.01 to 10% of the Et₃N-α-CHCA ionic liquid in acetonitrile as well as different volumes dopped into the spot (1–10 μL) have been tested. The best results were obtained for 3–5 μL of a 0.1%(w/v) acetonitrile solution.
(15) For the characterization of unknowns, multimode ionization is certainly not ideal, but the formation of both the ionized and protonated molecules seems to be a characteristic of the three alkaloids tested for on-spot MALDI. We tried to suppress or enhance formation of one ionic species over the other by varying the laser power and the ionic liquid (other acidic matrixes were tested), but little change in the relative abundances of both species was observed. Reducing the laser power trying to minimize formation of M⁺ caused drastic reduction in ion formation (sensitivity).

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Anesthetics. The ability to monitor different classes of compounds is crucial for the generality of the on-spot TMT-MS technique with an ionic liquid matrix as described herein. Figure 4 shows the TMT-MS spectra of levobupivacaine and mepivacaine. These two important anesthetics were separated by TLC and analyzed on-spot by MALDI-TOF MS after ionic liquid doping. Note the abundant, clearly detected ions of m/z 289 and 247 arising now from a single mode of ionization and corresponding to the protonated molecules of each anesthetic, with little matrix and background noise over a relatively large, low-m/z range. Interestingly, when solutions of these two anesthetics were doped into the same spot on the TLC, to mimic coelution, and the on-spot TMT-MS spectrum was acquired, the protonated molecules of both compounds were clearly detected (spectrum not shown). This result demonstrates the ability of the TMT-MS technique to characterize coeluting TLC analytes.

Antibiotics. An important antibiotic, tetracycline, has also been tested. Figure 5 shows its on-spot TMT-MS spectrum which, after background subtraction, displays predominantly the protonated molecule of m/z 445. Formation of mainly a single ion and, most particularly, the protonated molecule greatly benefits analyte MS identification. A previously reported TMT-MS mass spectrum of tetracycline using graphite particle suspension matrices and a TLC plate doped with EDTA displayed a more complex series of analyte ions: [M + K]+, [M + K – NH3]+, [M + Na – NH3]+, [M + Na – NH3 – H2O]+, and [M + H – NH3 – H2O]+ with no clear detection of the protonated molecule.

CONCLUSION

The effective application of TLC for low-MW compounds) with direct on-spot positive ion MALDI-TOF mass spectrometry identification using an UV-absorbing proton donor ionic liquid (Et3N-α-CHCA) as the matrix has been accomplished. Much reduced

(16) Since positive ion MALDI and a proton donor ionic liquid matrix were employed, emphasis has been given to the TMT-MS analysis of basic low-MW compounds. Acid low-MW compounds such as phenols and carboxylic acids should be better ionized by negative ion MALDI.

(17) For the tetracycline analysis, spectra subtraction from a side “blank” spot doped with the ionic liquid was beneficial likely due to coelution of impurities from the extracted material obtained from a commercial tablet.
matrix background and enhanced analyte detection was obtained as compared with previous TMT-MS methods. Our procedure uses no pretreatment of the TLC plate and requires no special composition of the TLC eluent. Previous TMT-MS analyses require the TLC plate to be sprayed with an aqueous disodium EDTA solution; or the use of TLC eluents doped with K⁺ or Na⁺ salts. A pressing method has also been applied in TMT-MS to transfer to the MALDI matrix zone the components present on the TLC spots via face-to-face pressing. In these methodologies, the analytes are also often detected in various cationic or fragmented forms. The simpler method herein described using the Et₃N·α-CHCA ionic liquid matrix with the proton donor species Et₃NH⁺ for basic low-MW compounds seems to favor the formation of a single ion and what most benefits MS identification, the protonated analyte molecule! 

(18) Although sensitivity should vary considerably with the analyte, for those tested here, clear mass spectra (a greater than 3 signal-to-noise ratio for the ionized or protonated molecule) were obtained for a limit of 5–10 ng/spot.

From the results described herein for selected proof-of-principle cases, the on-spot TMT-MS technique with the UV-absorbing Et₃N·α-CHCA ionic liquid matrix and with the proton donor species Et₃NH⁺ seems to be a relatively general, sensitive, fast screening method with MS identification for a variety of relatively low MW compounds and is likely to find applications in many areas, most particularly in phytochemistry and organic synthesis screening and manufacturing process quality monitoring.

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