Anal. Chem. **2004,** *76,* 2144−2147

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

Leonardo S. Santos,[†] Renato Haddad,[‡] Nelci F. Hϋehr,[‡] Ronaldo A. Pilli,[†] and Marcos N. Eberlin*,[†]

Thomson Mass Spectrometry Laboratory, Institute of Chemistry, and Faculty of Medical Science, State University of Campinas, UNICAMP, Campinas, SP Brazil

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 anesthesics levobupivacaine and mepivacaine, and the antibiotic tetracycline were readily characterized most frequently by the MS detection of their protonated molecules. 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 desortion/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 desortion/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 acccomplish 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,[6] drugs,[9] and pesticides[10] 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.[11]

In phytochemistry, organic synthesis, and manufacturing process quality monitoring, low-MW analytes are often the targets; hence, the use of MALDI matrixes 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.

* Corresponding author. Phone/Fax: (55-019)3788-3023. E-mail: eberlin@iqm.unicamp.br.

[†] Thomson Mass Spectrometry Laboratory, Institute of Chemistry.

[‡] Faculty of Medical Science.

(1) Karas, M.; Hillenkamp, F. *Anal. Chem.* **1988**, *60*, 2299.

(2) (a) Macfarlane, R. D.; Torgerson, D. F. *Science* **1976**, *191*, 920. (b) Barber, M.; Bordoli, R. S.; Sedgwick, R. D.; Tyler, A. N. *Nature* **1981**, *293*, 270.

(3) Zenobi, R.; Knochenmuss, R. *Mass Spectrom. Rev.* **1998**, *17*, 337.

(4) Zenobi, R. *Chimia* **1997**, *51*, 801.

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

(6) (a) Gusev, A. I.; Proctor, A.; Rabinovich, Y. I.; Hercules, D. M. *Anal. Chem.* **1995**, *67*, 1805. (b) Mehl, J. T.; Hercules, D. M. *Anal. Chem.* **2000**, *72*, 68. (c) Gusev, A. I.; Vasseur, O. J.; Proctor, A.; Sharkey, A. G.; Hercules, D. M. *Anal. Chem.* **1995**, *67*, 4565. (d) Nicola, A. J.; Gusev, A. I.; Hercules, D. M. *Appl. Spectrosc.* **1996**, *50*, 1479. (e) Mehl, J. T.; Gusev, A. I.; Hercules, D. M. *Chromatographia* **1997**, *46*, 358.

(7) Isbell, D. T.; Gusev, A. I.; Taranenko, N. I.; Chen, C. H.; Hercules, D. M. *Fresenius J. Anal. Chem.* **1999**, *365*, 625.

(8) Matsumoto, K.; Ajiro, H.; Habaue, S.; Okamoto, Y. *J. Mass Spectrom. Soc. Jpn.* **2001**, *49*, 127.

(9) Crecelius, A.; Clench, M. R.; Richards, D. S.; Parr, V. *J. Chromatogr., A* **2002**, *958*, 249.

(10) Vermillion-Salsbury, R. L.; Hoops, A. A.; Gusev, A. I.; Hercules, D. M. *Int. J. Environ. Anal. Chem.* **1999**, *73*, 179.

(11) Mowthorpe, S.; Clench, M. R.; Cricelius, A.; Richards, D. S.; Parr, V.; Tetler, L. W. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 264.

10.1021/ac035387d CCC: $27.50
Published on Web 03/09/2004

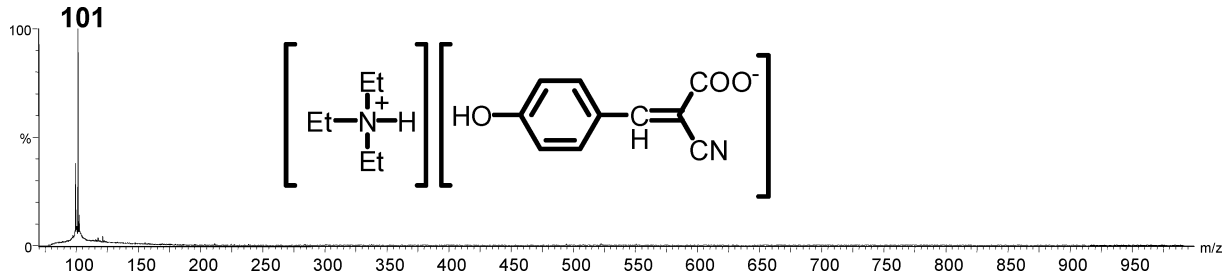

Figure 1. On-spot TMT-MS mass spectrum acquired directly from a blank TLC spot dopped with the Et_3N·α-CHCA ionic liquid matrix. Note that the matrix produces upon positive ion MALDI a nearly exclusive single ion of *m*/*z* 101, that is, Et_3NH^+.

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 (Et_3N·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 vaccum. MALDI mass spectra were acquired on a Micromass MALDI-TOF instrument in the reflectron mode. The main settings were as follows: pulse voltage, 2450 V; delay extraction, 100 ns; acelerating 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 Et_3NH^+ 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[12] has demonstrated the suitability of ionic liquids as MALDI matrixes. To test an ionic liquid as a suitable matrix for TMT-MS, we first collected a mass spectrum on a blank TLC spot dopped with 1 μL of the Et_3N·α-CHCA matrix (Figure 1). Nearly, just a single ion from the matrix was detected by positive ion MALDI-MS: Et_3NH^+ of *m*/*z* 101.

Alkaloids. TMT-MS was then performed for a mixture of three alkaloids previously synthesized and characterized as arborescidines A−C.[13] Figure 2 shows the TLC plate resting on a MALDI sample plate with three colorful spots. Each of these spots corresponds to the three alkaloids separated by TLC from a 10 mg/L chloroform solution of the mixture and by applying 0.2 mL of such solution on the TLC plate (aproximately 1−2 μg) and eluting with $CHCl_3$/MeOH 9:1 with no previous treatment of the commercial TLC plate. Note that the spots, which after TLC separation originally displayed an oval shape, have been developed to a circular shape due to matrix application (on the center of the spot). However, nearly no radial expansion occurred across the TLC development axis so TLC perfornance was not degraded. When on-spot MALDI-MS analysis was perfomed with no matrix addition, no analyte ions were identified in the mass spectra. But after doping each spot with 3−5 μL of a 0.1% (w/v) acetonitrile solution of the Et_3N·α-CHCA ionic liquid,[14] followed by high vacum

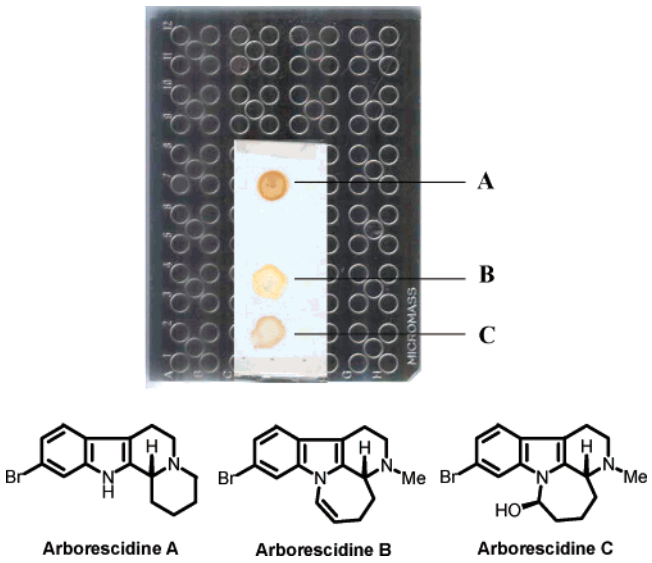

Figure 2. TLC separation of a mixture of three alkaloids A−C.

solvent evaporation, efficient ionization occurs with little non-interfering matrix noise.

As Figure 3 shows as an example for arborescidine C, the on-spot TMT-MS spectrum shows ions of great abundance arising from analyte ionization. The ions of *m*/*z* 333, 335, and 337 are easily rationalized[3] 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.[15] 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-MS analysis of the other two alkaloids A and B afforded similar mass spectra (not shown), with abundant $M^{\bullet+}$ and MH^+ ions and nearly undetectable matrix background ions.

(12) Armstrong, D. W.; Zhang, L.-K.; He, L. F.; Gross, M. L. *Anal. Chem.* **2001**, *73*, 3679.

(13) Santos, L. S.; Pilli, R. A.; Rawal, V. H. *J. Org. Chem.* **2004**, *69*, 1283.

(14) Different concentrations ranging from 0.01 to 10% of the Et_3N·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 $M^{+\cdot}$ caused drastic reduction in ion formation (sensitivity).

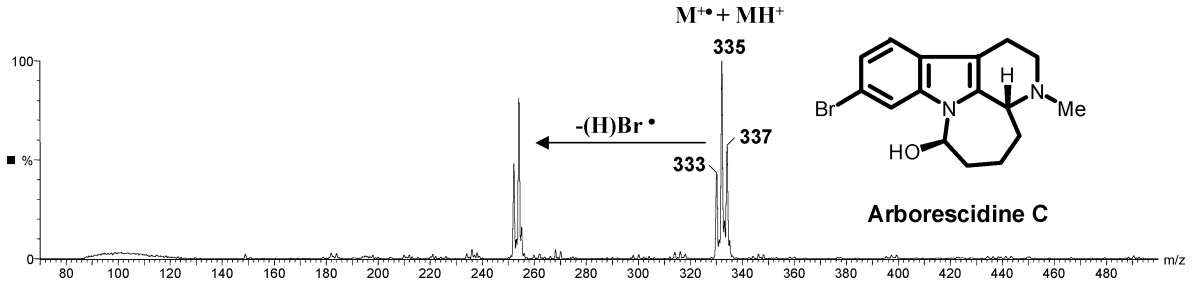

Figure 3. On-spot TMT mass spectrum of arborescidine C.

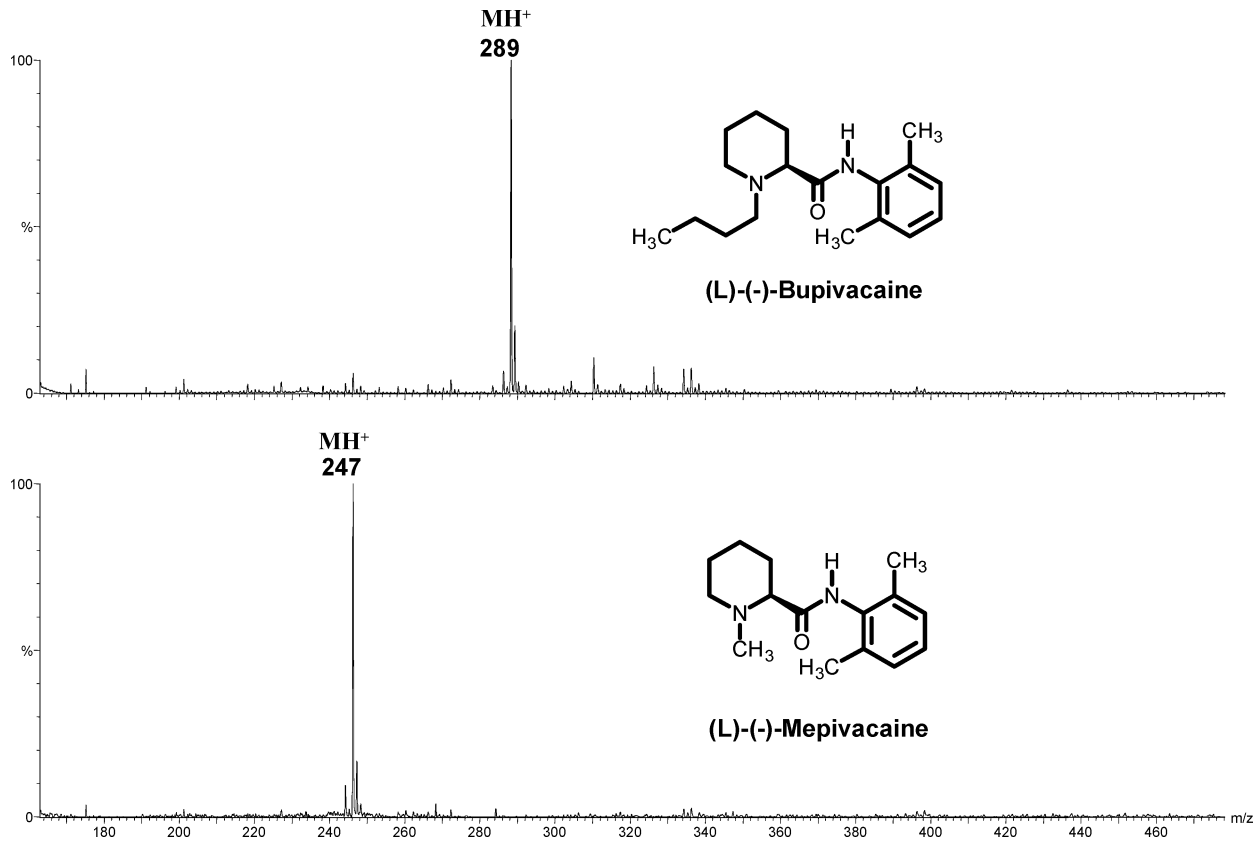

Figure 4. On-spot TMT-MS spectra of (a) levobupivacaine and (b) mepivacaine.

Anesthetics. The ability to monitor different classes of compounds[16] 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 the these two anhestesics were dopped 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.[16] Figure 5 shows its on-spot TMT-MS spectrum which, after background subtraction,[17] 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 matrixes and a TLC plate doped with EDTA displayed a more complex series of analyte ions: $[M + K]^+$, $[M + K - NH_3]^+$, $[M + Na - NH_3]^+$, $[M + Na - NH_3 - H_2O]^+$, and $[M + H - NH_3 - H_2O]^+$ with no clear detection of the protonated molecule.[9]

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 (Et_3N·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.

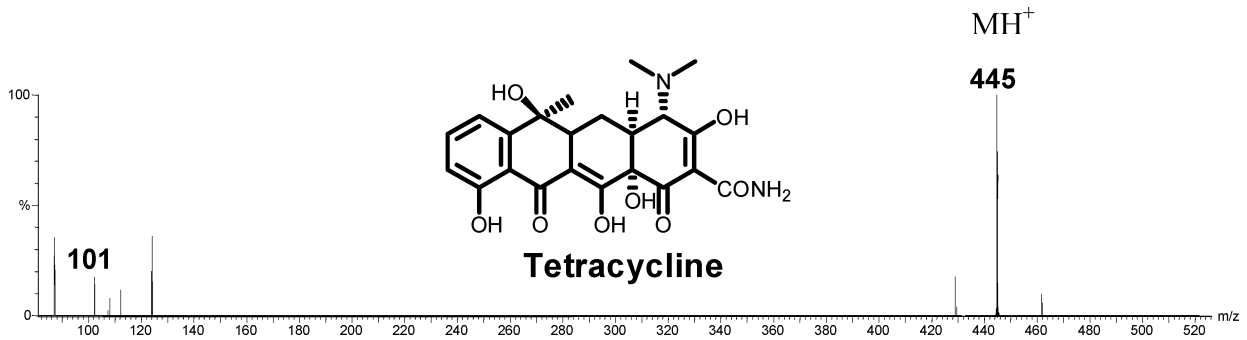

Figure 5. On-spot TMT-MS spectrum of tetracycline after background spectra subtraction.

matrix background and enhanced analyte detection was obtained as compared with previous TMT-MS methods.[6–11] 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;[9] or the use of TLC eluents doped with K^+ or Na^+ salts.[5] 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.[6] In these methodologies, the analytes are also often detected in various cationic or fragmented forms. The simpler method herein described using the Et_3N·α-CHCA ionic liquid matrix with the proton donor species Et_3NH^+ for basic low-MW compounds seems to favor the formation of a single ion and what most benefits MS identification, the protonated analyte molecule!

From the results described herein for selected proof-of-principle cases, the on-spot TMT-MS technique with the UV-absorbing Et_3N·α-CHCA ionic liquid matrix and with the proton donor species Et_3NH^+ seems to be a relatively general, sensitive,[18] 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.

ACKNOWLEDGMENT

Financial support by the Research Support Foundation of the State of São Paulo (FAPESP) and the Brazilian National Research Council (CNPq) is acknowledged.

Received for review November 22, 2003. Accepted January 12, 2004.

AC035387D

(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.