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Easy Ambient Sonic-Spray Ionization Mass Spectrometry Combined with Thin-Layer Chromatography

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On-spot detection and analyte characterization on thin-layer chromatography (TLC) plates is performed via ambient desorption/ionization and (tandem) mass spectrometry detection, that is, via easy ambient sonic spray ionization mass spectrometry (EASI-MS). As proof-of-principle cases, mixtures of semipolar nitrogenated compounds as well as pharmaceutical drugs and vegetable oils have been tested. The technique has also been applied to monitor a chemical reaction of synthetic importance. EASI is the simplest and gentlest ambient ionization technique currently available, assisted solely by N_2 (or air). It uses no voltages, no electrical discharges; no UV or laser beams, and no high temperature and is most easily implemented in all API mass spectrometers. TLC is also the simplest, fastest, and most easily performed chromatographic technique. TLC plus EASI-MS therefore provide a simple and advantageous combination of chromatographic separation and sensitive detection of the TLC spots as well as on-spot MS or MS/MS characterization. The favorable characteristics of TLC-EASI-MS indicate advantageous applications in several areas such as drug and oil analysis, phytochemistry and synthetic chemistry, forensics via reliable counterfeit detection, and quality control.

Ambient mass spectrometry,[1] that is, the desorption, ionization, and characterization via mass spectrometry of an analyte (or mixture of analytes) directly from their natural matrixes via a nonsample preparation procedure under atmospheric pressure and room temperature, is one of the most-welcomed advances in modern mass spectrometry. This previously unimaginable feature of MS was made possible via the continuous efforts of many mass spectrometrists in developing a myriad of ionization techniques, which culminated recently with the introduction of several ambient ionization techniques. These innovative techniques include desorption electrospray ionization,[2] direct analysis in real time,[3] analysis of samples at atmospheric pressure,[4] desorption atmospheric pressure photonionization,[5] and desorption sonic spray ionization,[6] a technique that was recently renamed as EASI (easy ambient sonic spray ionization).

Among these techniques, EASI is the simplest, gentlest, and most easily implemented. It requires no (high) voltage, no UV lights, no laser beams, no corona or glow discharges, and no heating. An EASI source can be constructed and installed in a few minutes from a few simple MS laboratory parts (see Figure 1) and is assisted only by compressed N_2 or air.[7] EASI uses the gentlest sonic spray ionization (SSI)[8] to create charged droplets, which are formed due to the (super) sonic spray that causes a statistical imbalance distribution of charges. EASI has been applied with success to the analysis of different analytes and matrixes such as drug tablets,[6] oils,[9] perfumes,[10] and wine[11] and has been recently coupled to membrane introduction mass spectrometry[12] using cellulose membranes for EASI-MIMS ambient ionization of polar analytes from the surface of solutions.[13]

Thin-layer chromatography (TLC) is also among the simplest, least expensive, and most easily performed chromatographic methods for mixture analysis. It displays, however, relatively low resolution and poor detection limits. But TLC separation efficiency can be significantly improved by applying two-dimensional (2D) elution or using modern HPTLC and new application techniques.[14] Several MS strategies have also been shown to provide improved sensitiv-

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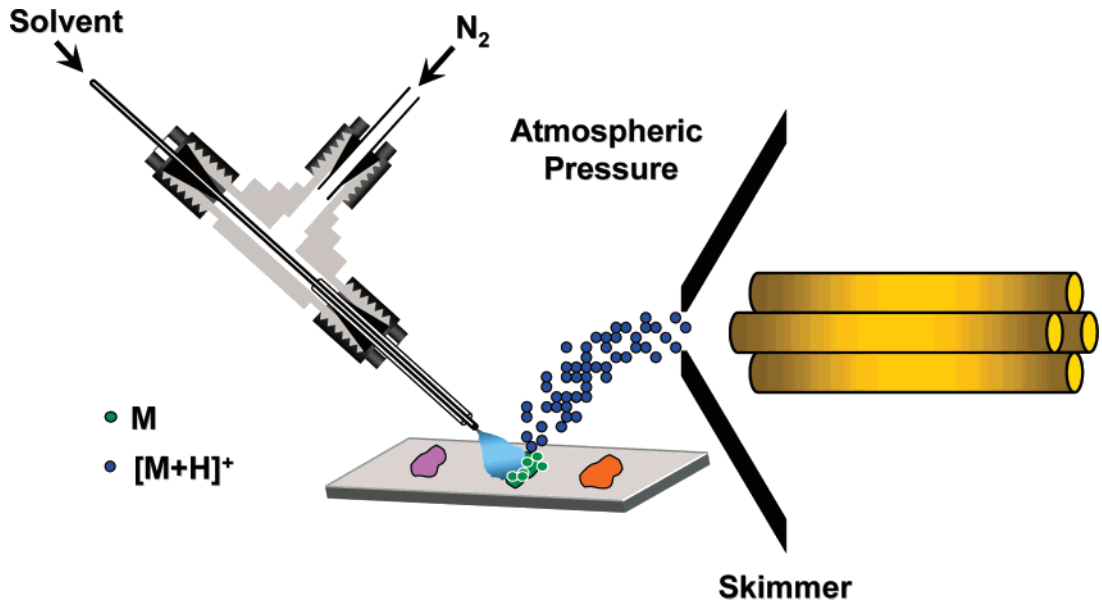

Figure 1. Schematic of the TLC-EASI-MS system in operation. The desorption/ionization of the analytes from the surface of the TLC plates is performed by the charged droplets produced by sonic spraying an acidic water/methanol solution in a N_2 (or air) assisted process. The charged droplets are created during sonic spray by a statical imbalance of charges. The EASI source uses only a Swagelok T-element, ferrules, and tubing for the gas flow and a fused-silica capillary at the sonic spray exit.

ity for TLC detection and superior *on-spot* MS characterization.[15] TLC also provides a proper surface in which direct on-spot desorption and ionization of analytes can be performed. Ambient mass spectrometry techniques are therefore likely to provide the most effective way to perform this task, as has already been demonstrated.[15].

Herein we report the coupling of the simplest ambient ionization MS technique with the simplest chromatographic method. We show that EASI-MS is able to provide fast, selective, and sensitive on-spot TLC detection and characterization operating in both the MS and MS/MS modes. This combination leads to a hybrid technique with many potential and advantageous applications.

EXPERIMENTAL SECTION

Chemicals. Formic acid and HPLC-grade methanol and ethyl acetate (EtOAc) were purchased from Merck SA (Rio de Janeiro, Brazil) and used without further purification. Deionized water was obtained from a MilliQ (Millipore, Billerica, MA) purification unit. Commercial TLC aluminum sheets were used (0.25-mm silica gel with fluorescent indicator, Alugram SIL G/UV254, Macherey-Nagel). All reagents and solvents were obtained from commercial sources.

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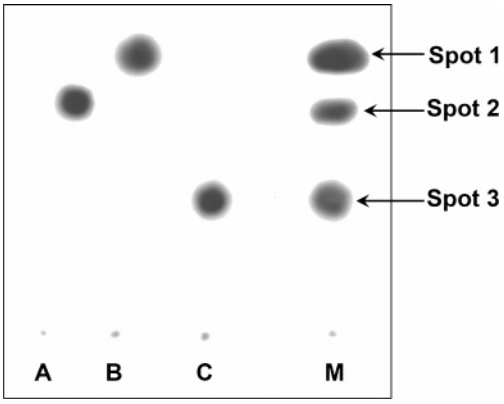

Figure 2. TLC of each of the 3 test molecules: (A) allyl phenylamine, (B) phenylamine, and (C) ethylpyridine and for the A−C mixture (M).

EASI-MS. MS experiments were performed on a Q-Trap triple-quadrupole mass spectrometer (Applied Biosystems) equipped with a homemade EASI source mounted on the supplied nano-ESI source.[16] This EASI-MS system is described in detail elsewhere.[17] The principal experimental parameters were as follows: flow rate of the 1:1 acidic (0.01% formic acid) water/methanol solution of 20 μL min^{-1}, nebulizing gas back pressure of ~30 bar, curtain gas pressure of 5 bar, declustering potential of 100 V, tip-TLC plate distance of ~2 mm, and capillary-TLC plate-entrance angle of ~30°.

RESULTS AND DISCUSSION

Test Mixture of Semipolar Compounds. To test the performance of TLC-EASI-MS for on-spot detection and characterization,

(16) We mounted the EASI source on the commercial AB nanospray ESI source for convenience only, for the Qtrap instrument would not work properly without one of its commercial sources connected to it. However, any simple holder should work as well for proper EASI operation.

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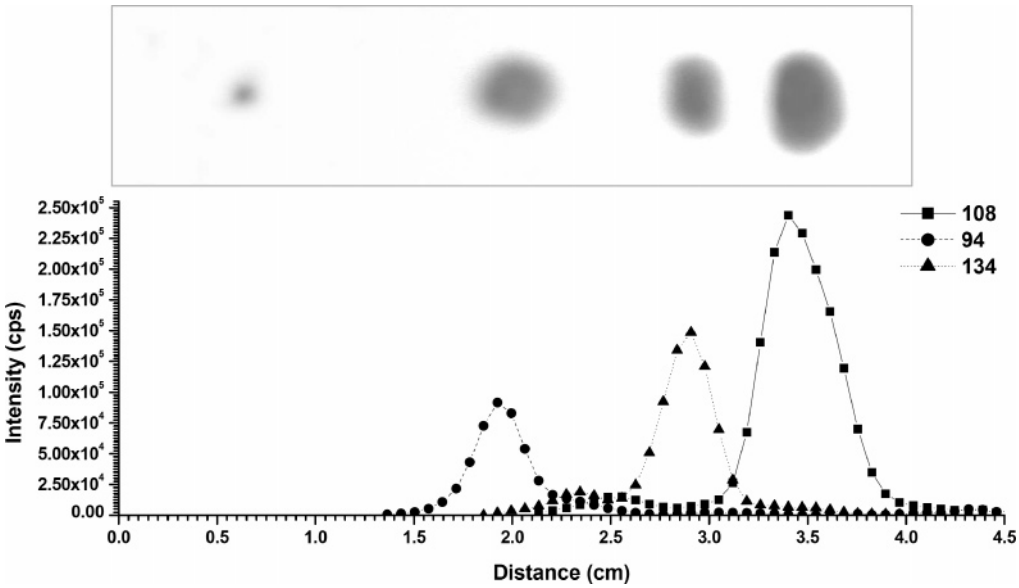

Figure 3. SIM for EASI-MS revelation of TLC of compounds A−C: (■) *m/z* 108, (●) 134, and (▲) 94.

an equimolar mixture of three semipolar nitrogenated molecules was employed, that is, allyl phenylamine (A), phenylamine (B), and ethylpyridine (C) (Figure 2). The mixture of these simple molecules was selected as a test case since they are resolved properly by TLC, produce rather visible spots via UV detection, and are efficiently ionized by sonic spray ionization. TLC was performed by applying ~1 μL of a 0.1 mg mL^{-1} in ethyl acetate solution of each component and by eluting with EtOAc/hexane (2:8).

TLC Detection. As Figure 3 shows, EASI-MS can be used with confidence to reveal TLC plates (at least when semipolar to polar components are used and both modes of ionization, negative and positive ion modes, are applied), particularly to double check or expand UV detection. Using either (a) total ion current in the full 50−300 *m/z* range or (b) selective ion monitoring (SIM) for each protonated molecule, the three UV spots are detected as intense peaks.

MS Characterization. By far the greatest advantage of using MS detection of TLC spots is structural characterization. Figure 4 displays the full on-spot EASI-MS for the three separated components of the mixture as well as for the TLC background. For all three mass spectra, the background ions are suppressed and clean and intense mass spectra are recorded. All these spectra show a major ion corresponding to the protonated molecule. The ions of *m/z* 106 and 93 in the EASI-MS of spot 1 correspond to minor fragments of the protonated molecule of *m/z* 134.

EASI is based on SSI, and SSI has been shown to be the softest ionization technique.[18] This gentleness is another advantage of EASI since its superior ability to form intact protonated (or deprotonated) molecules with little or no dissociation should greatly simplify the detection of TLC coelution or cross-contamination. This feature is nicely illustrated by the EASI-MS of the intermediate spot 2. It shows a prominent ion of *m/z* 94 due to protonated aniline, but the detection of the minor ion of *m/z* 134 indicates that phenylaniline is also present in spot 2. Hence, although UV detection indicates clear separation of A and B, the EASI-MS indicates some residual A in spot 2 due to a minor tail, which is nearly invisible via UV detection. Note that the B tail is also visible via SIM of the ion of *m/z* 134 in Figure 3.

Drug Tablets. The application of TLC-EASI-MS for the fast screening of drug tablets was tested for two samples: propranolol hydrochloride (propranolol) and amlodipine besylate (Norvasc). The commercial drug tablets were ground, dissolved in 10.0 mL of purified water, neutralized with ammonium hydroxide (pH 8.0), and extracted with 5 mL of ethyl acetate. Then, 0.05 mL of this solution was applied to a TLC plate for analysis. A clean TLC spot was observed for each drug (not shown), and Figure 5a shows the on-spot TLC-EASI-MS of propranolol. Note the intense ion of *m/z* 260 for $[M + H]^+$ and *m/z* 282 for $[M + Na]^+$. Figure 5b shows, for amlodipine, the on-spot TLC-EASI-MS/MS of its $[M + H]^+$ ion of *m/z* 409. Note the fragments of *m/z* 294 and 238, which are characteristic of this drug.[6] Although most drug tablets can be analyzed directly by EASI-MS with no chromatographic separation,[6] fast TLC may be beneficial for multidrug tablets (to avoid ion suppression) or for forensic fingerprinting analysis of counterfeit tablets[19] to characterize known and unknown impurities.

Reaction Monitoring. A straightforward and advantageous application of TLC EASI-MS would be for monitoring chemical reactions. Proper transformation of reactants into intermediates and final products could be monitored, and unexpected intermediate or side product detected (by the gentlest possible desorption/ionization method) can be characterized by either MS or MS/MS. To illustrate this interesting application, we monitored a Ritter-type reaction[20] used to convert oxiranecarboxylic esters into

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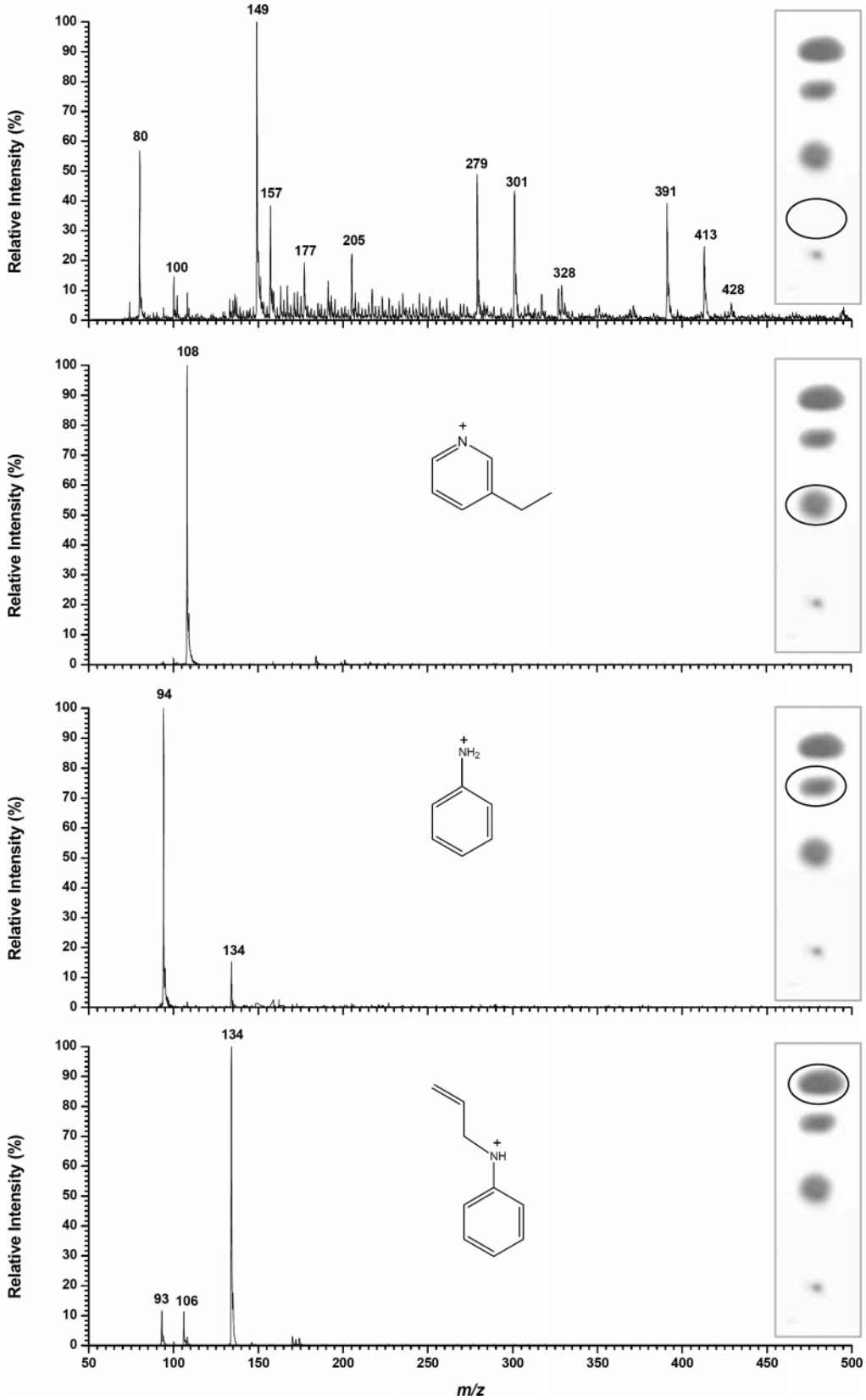

Figure 4. On-spot TLC-EASI-MS for the background and for the three separated components.

oxazoline-5-carboxylic esters (Scheme 1). This important reaction is used, for instance, in the synthesis of *syn*-3-amino-2-hydroxy esters during construction of lateral chains in the taxol synthesis.[21]

$BF_3.Et_2O$ (0.2 mL) was added to an acetonitrile solution of the oxirane (0.3 mmol) in anhydrous acetonitrile under argon, and the reaction was kept at room temperature for 4 h.[22] For the

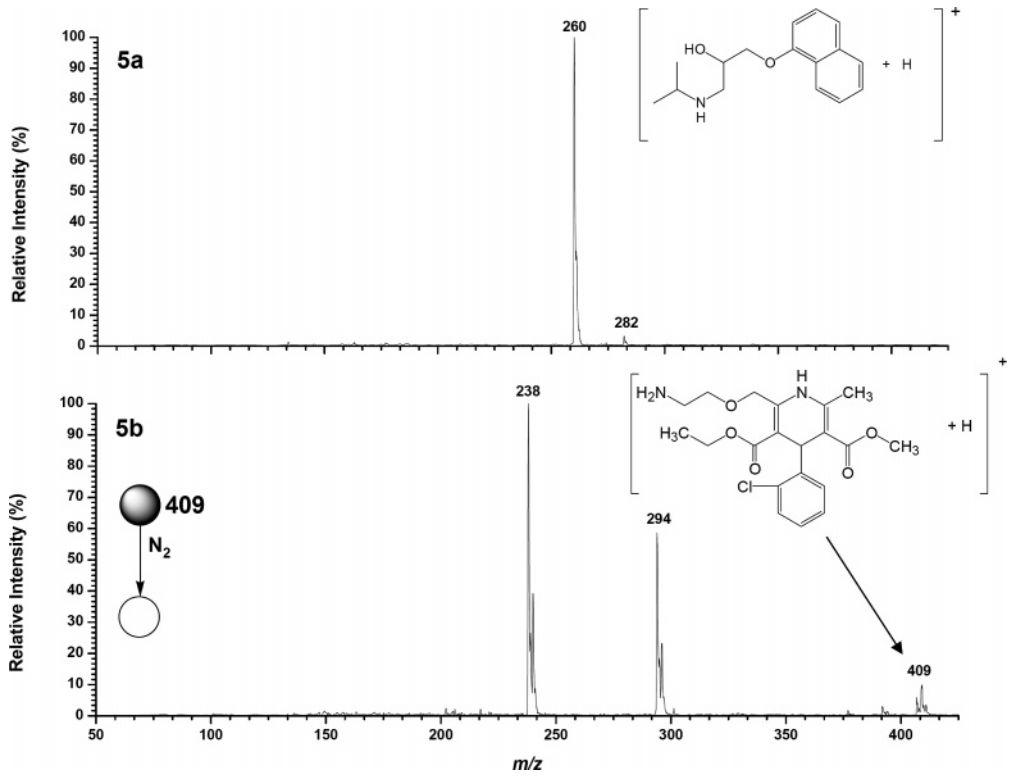

Figure 5. TLC-EASI-MS of (a) propranolol and (b) TLC-EASI-MS/MS of amlodipine.

Scheme 1

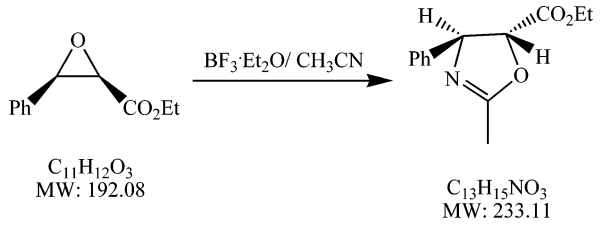

Table 1. Principal TAG Identified by TLC-EASI-MS of Olive Oil

elemental composition	TAG[a]	CN/DB[b]	$[M + H]^+$	$[M + Na]^+$ [c]
$C_{53}H_{100}O_6$	PPO	53:1	834	856
$C_{55}H_{100}O_6$	PLO	55:3	857	879
$C_{55}H_{102}O_6$	POO	55:2	860	881
$C_{55}H_{104}O_6$	SOP	55:1	861	883
$C_{57}H_{98}O_6$	LLL	57:6	879	901
$C_{57}H_{100}O_6$	LLO	57:5	881	903
$C_{57}H_{102}O_6$	LOO	57:4	883	905
$C_{57}H_{104}O_6$	OOO	57:3	885	907
$C_{57}H_{106}O_6$	OOS	57:2	887	909

[a] Triglycerol abbreviations: PPO,1,2-dipalmitoyl-3-oleylglycerol; PLO, 1-palmitoyl-2-linoleyl-3-oleylglycerol; POO, dioleyl-1-palmitoilglycerol; SOP, 2-oleyl-3-palmitoyl-1-stearoylglycerol; LLL, 1,2,3-trilinoleylglycerol; LLO, 1,2-dilinoleoyl-3-oleylglycerol; LOO, 2,3-dioleyl-1-linoleyglycerol; OOO, 1,2,3-trioleoylglycerol; OOS, 1,2-dioleoyl-3-stearoylglycerol, and their isomers. [b] Carbon number: number of double bonds. [c] Note the occurrence of *m/z* overlaps between some $[M + H]^+$ and $[M + Na]^+$ ions.

TLC-EASI-MS monitoring, samples were collected at time zero, and after 2 and 4 h of reaction. TLC was done with 50% ethyl acetate in hexane.

Table 2. Principal DAG Identified by TLC-EASI-MS of Olive Oil

elemental composition	DAG	CN/DB	$[M - H_2O + H]^+$
$C_{39}H_{72}O_5$	OO	39:2	603
$C_{37}H_{70}O_5$	OP	37:1	595
$C_{35}H_{68}O_5$	PP	35:0	551

Figure 6 shows the TLC and respective EASI-MS for the reaction monitoring. At time zero, TLC via UV detection shows a single spot corresponding to the starting epoxide reactant. EASI-MS on this spot detects the reactant epoxide mainly via its $[2M + NH_4]^+$ dimer of *m/z* 402. After 4 h of reaction, the synthesis is practically completed and the final product is detected by a single TLC spot and an intense ion in the EASI-MS corresponding to the protonated oxazoline of *m/z* 234. In the intermediate stage of the reaction (2 h), TLC shows two major spots (as revealed by UV) corresponding to the starting epoxide and final oxazoline product, as shown by the corresponding EASI-MS that detects both ions of *m/z* 234 and 402.

Oil Analysis. As a representative example, EtOAc solutions of several vegetable oils were subjected to TLC with 100% EtOAc as the eluent. Figure 7 shows, as an example, the on-spot EASI-MS for the olive oil spot. Note that both the diglycerides (DAG) and triglycerides (TAG) coeluted and are therefore detected in

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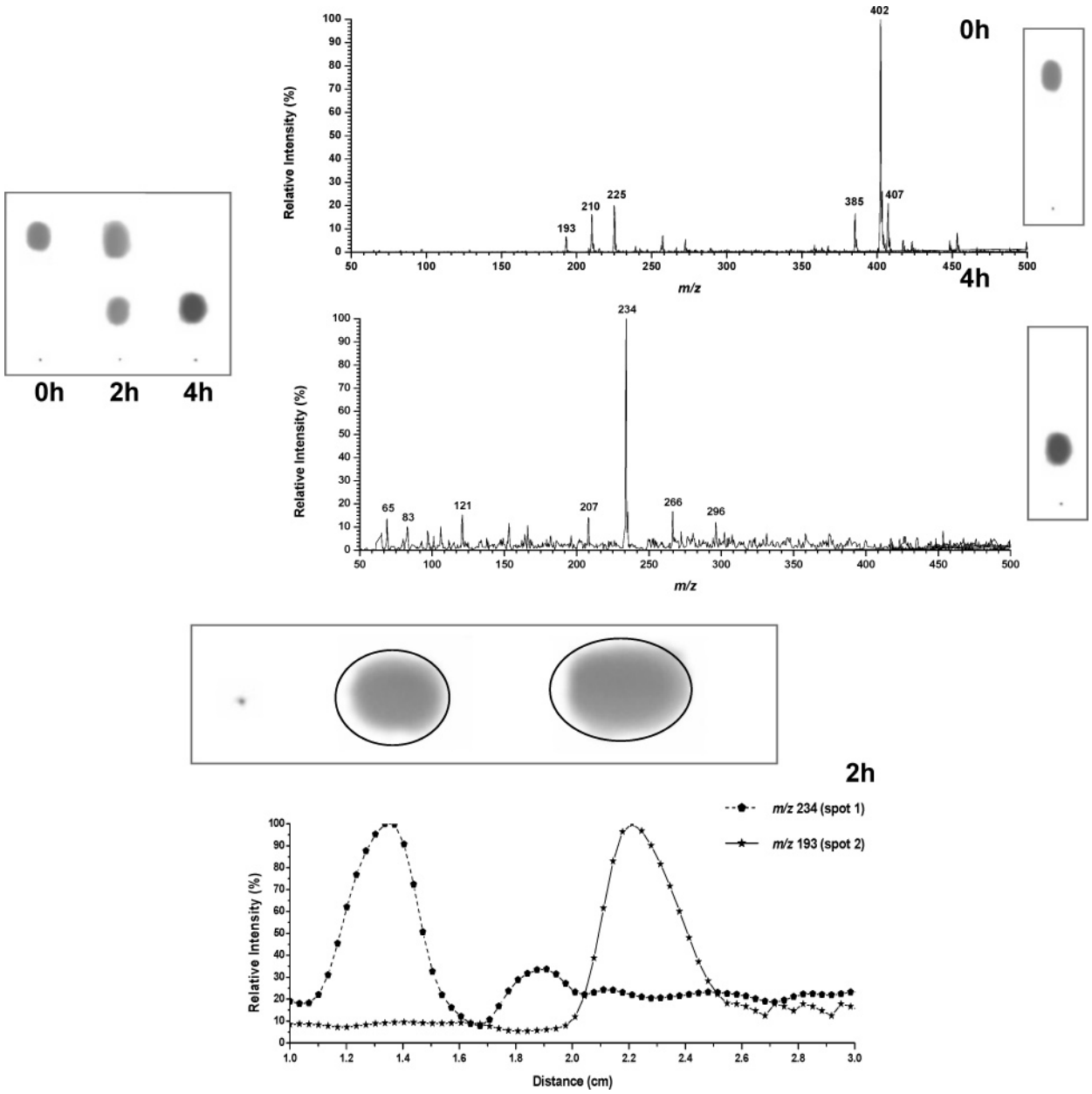

Figure 6. On-spot TLC-EASI-MS monitoring of the synthesis of oxazoline-5-carboxylic ester.

the form of both their protonated and sodiated molecules, that is, $[M + H]^+$ and $[M + Na]^+$, as summarized in Tables 1 and 2. This experiment shows that EASI-MS is able to typify oil spots after TLC separation. This interesting ability of TLC-EASI-MS is currently being explored in our laboratory to, for instance, separate, characterize and quantify biodiesel fatty esters[23] from their most common impurities, that is, residual DAG and TAG (from the parent vegetable or animal oil) and glycerine (the transesterification coproduct). We are also investigating the application of this fast methodology to quantify diesel/biodiesel mixtures and for fast separation and identification of oils when present in complex mixtures such as those found in food products and living organisms.

CONCLUSION

The advantageous combination of TLC with EASI-MS has been demonstrated. TLC-EASI-MS is shown to provide the simplest and most easily implemented technique for the separation of mixtures followed by sensitive, gentle, and selective on-spot detection and MS or MS/MS characterization. As briefly demonstrated herein, TLC-EASI-MS/MS can be readily and advantageously applied (by anyone having access to a mass spectrometer that uses atmospheric pressure ionization) to many analytical tasks such as drug and oil analysis, reaction monitoring, phytochemistry and synthetic chemistry, forensic counterfeit screening, and quality control.

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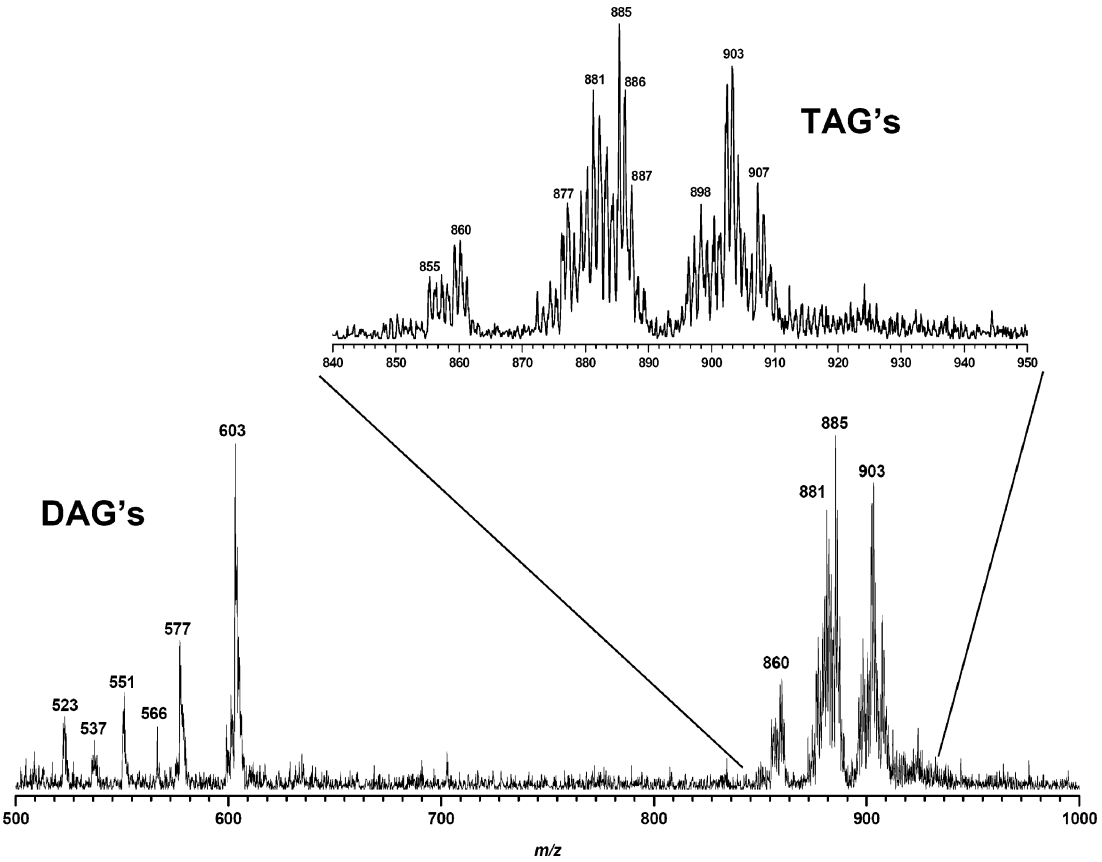

Figure 7. On-spot TLC-EASI-MS of olive oil. Note the detection of both DAG and TAG in the form of their protonated or sodiated molecules (See Tables 1 and 2).

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