ORGANIC LETTERS

2008
Vol. 10, No. 16
3493−3496

HRMS Directly From TLC Slides. A Powerful Tool for Rapid Analysis of Organic Mixtures

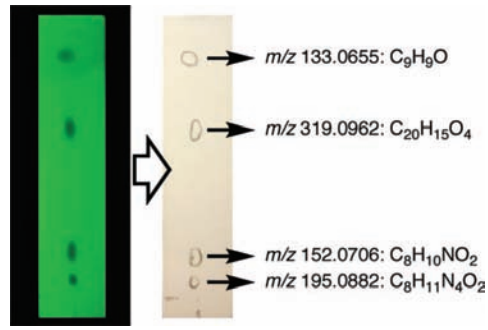
Natalie J. Smith, Marek A. Domin, and Lawrence T. Scott*

Department of Chemistry, Merkert Chemistry Center, Boston College, Chestnut Hill, Massachusetts 02467-3860

lawrence.scott@bc.edu

Received June 5, 2008

ABSTRACT



High-resolution mass spectra (HRMS) of individual spots on thin-layer chromatography (TLC) slides can now be obtained quickly and easily at atmospheric pressure, with zero sample preparation, using commercially available instrumentation. The method is complementary to GC−mass spectrometry but is not limited to compounds of high volatility and high thermal stability. TLC−HRMS can be used to monitor chemical reactions in real time and has the capacity thereby to accelerate significantly the pace of synthetic organic chemistry.

Thin-layer chromatography (TLC) continues to enjoy widespread popularity as one of the fastest and simplest methods for monitoring the progress of reactions in synthetic organic chemistry.[1] Disappearance of the spot corresponding to starting material on consecutive TLC slides is generally accompanied by the appearance of one or more new spots corresponding to the product(s). Assigning chemical structures to the products that give rise to each new spot typically entails preparative TLC, HPLC, or column chromatography for isolation of the various components, which are then analyzed individually by NMR and/or other spectroscopic methods. By using the new open air ionization sources recently developed for mass spectrometry,[2] however, it is now a simple matter to obtain high-resolution mass spectra (HRMS) of compounds directly from standard TLC slides, routinely, *even while monitoring the course of a chemical reaction*, without the need for preparative-scale chromatography or time-consuming sample preparation. Herein we illustrate the simplicity and power of this new tool by describing the TLC separation and direct HRMS analysis of the components in an artificial mixture of four familiar organic compounds: cinnamaldehyde, phenolphthalein, acetaminophen, and caffeine (see abstract graphic and Figure 1).[3]

The four spectra pictured in Figure 1 show base peaks for the individual compounds as their protonated molecular ions

(1) (a) Sherma, J. *Anal. Chem.* **2000**, *72*, 9R–25R. (b) Calimente, D. S.; Strand, S. M.; Chang, S.; Lewis, D. E. *J. Chem. Educ.* **1999**, *76*, 82–83. (c) Jones, T. B.; Jones, T. H. *J. Chem. Educ.* **1985**, *62*, 813–814. (d) *Introduction to Organic Laboratory Techniques: A Microscale Approach*, 3rd ed.; Pavia, D. L., Lampman, G. M., Kriz, G. S., Engel, R. G., Eds.; Brooks/Cole-Thomson Learning: Pacific Grove, CA, 1999; pp 115−119.

(2) (a) McEwen, C.; Gutteridge, S. *J. Am. Soc. Mass Spectrom.* **2007**, *18*, 1274–1278. (b) Takáts, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G. *Science* **2004**, *306*, 471–473. (c) Cotte-Rodriguez, I.; Takáts, Z.; Talaty, N.; Chen, H.; Cooks, R. G. *Anal. Chem.* **2005**, *77*, 6755–6764.

10.1021/ol8012759 CCC: $40.75
Published on Web 07/16/2008

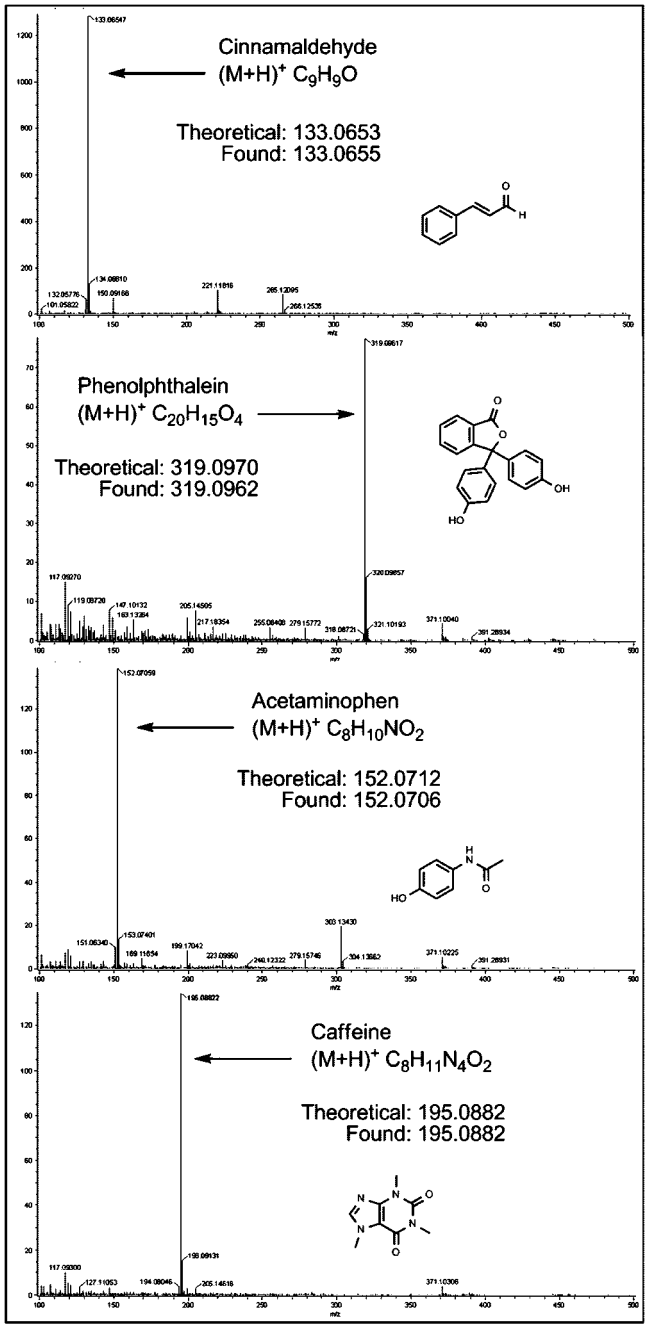


Figure 1. High-resolution mass spectra recorded directly from the four spots on the TLC slide shown in the abstract graphic. The spectra are pictured in the same order, top to bottom, as the TLC spots from which they were taken.

(M + 1 peaks). In our experience, some compounds also show small peaks corresponding to proton-bound dimers (2M + 1 peaks), e.g., cinnamaldehyde and acetaminophen.[4]

In 2005, the DART (direct analysis in real time) ionization source was introduced for use with standard time-of-flight mass spectrometers.[5] This atmospheric pressure ionization method uses a hot stream of excited-state helium atoms to excite clusters of water molecules in the atmosphere, which in turn transfer protons to the molecules of interest, thus creating the organic ions. Producing a spectrum requires only that the sample be held momentarily in the gas stream (more details below). Computer software compares the data to a calibration standard to yield precise mass measurements.

Because DART is a "soft ionization" method, the compounds chosen for this illustration all give simple mass spectra consisting of only one or two peaks. A mixture of these compounds in methanol was spotted on a plastic backed silica TLC slide,[6] and the slide was developed with 35% hexanes/65% ethyl acetate as the eluant. The four compounds separated completely from each other, as shown in the abstract graphic.

Figure 2. Holding the TLC slide in the DART mass spectrometer.[8]

(3) The mass spectrometer used in this work is a standard, commercial, single reflectron time-of-flight instrument with a resolving power of 6000 (at full-width-half-maximum), a sensitivity of S/N >10 (10 pg of reserpine), and a mass accuracy of 5 ppm (rms). Standard factory settings were used for the ionization source: needle voltage 3500 V; discharge electrode voltage 150 V; grid electrode voltage 250 V; heater control 250 °C. Normal settings were used on the mass analyzer: peaks voltage 1000 V; detector voltage 2400 V.

(4) (a) Marotta, E.; Paradisi, C. *J. Mass Spectrom.* **2005**, *40*, 1583–1589. (b) Ewing, R. G.; Eiceman, G. A.; Stone, J. A. *Int. J. Mass Spectrom.* **1999**, *193*, 57–68.

(5) Cody, R. B.; Laramée, J. A.; Durst, H. D. *Anal. Chem.* **2005**, *77*, 2297–2302.

(6) The TLC slides used in this work were standard, commercially available 200 μm silica gel on polyester backing with UV254 indicator. Some of the small background peaks seen in the mass spectra probably arise from the fluorophore and/or the plastic backing and may vary from one manufacturer to another.

For mass spectral analysis, the spots were visualized under a UV lamp and circled with a pencil.[7] The TLC slide was then cut with scissors down the center of the four spots, and the edge of the TLC slide was held[8] so that the gas stream grazed the center of each spot (Figure 2), beginning at the bottom of the slide and ending with the compound that traveled the furthest.

The slide was removed from the gas stream for a few seconds between each spot to clearly separate the peaks in the resulting ion count vs time data file (Figure 3).

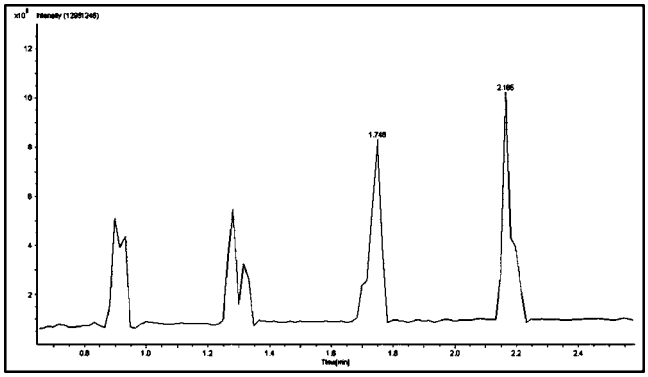


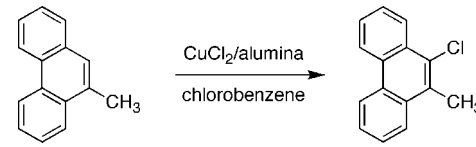
Figure 3. Ion count vs time data file. Total ion counts are recorded by the spectrometer only when samples are placed in the ionizing beam.

A solution of polyethylene glycol (average molecular weight: 600 g/mol) in methanol was used as the calibration standard for HRMS determinations. To provide a background spectrum of the TLC slide for automatic subtraction from the analyte spectra, a region of the cut TLC slide that was clear from any of the four compounds of interest was also held in the gas stream for a few seconds. Exercising this background correction was found to give cleaner, more accurate spectra of the separated compounds.

This same technique has been successfully used in our laboratory to monitor synthesis reactions, such as the chlorination of 9-methylphenanthrene by the Kodomari procedure (Scheme 1).[9] The progress of the reaction was checked periodically by TLC. Direct analysis of the TLC spots by DART mass spectrometry gave the high resolution spectra shown in Figure 4. It is noteworthy that even unfunctionalized aromatic hydrocarbons and their halogenated derivatives show up readily under standard DART conditions.

Some molecules require elevated temperatures for vaporization and ionization; for these, the gas stream can be heated

Scheme 1. Chlorination of 9-Methylphenanthrene



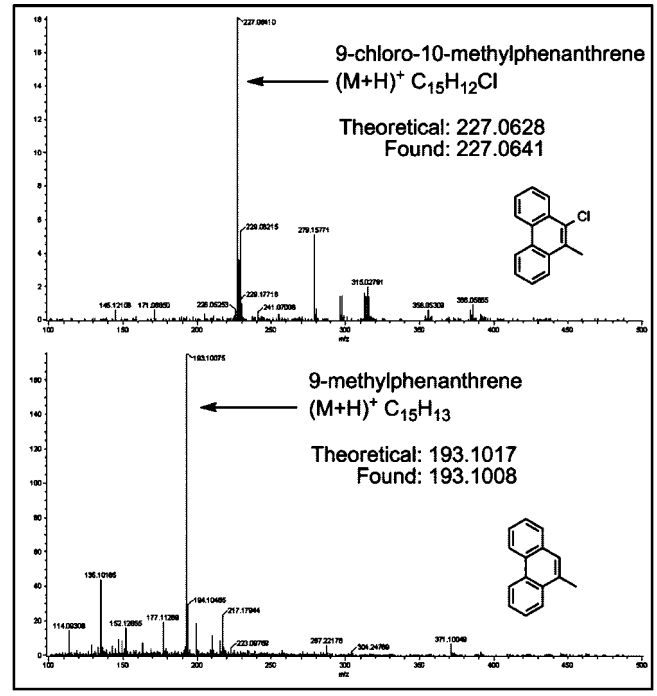


Figure 4. High-resolution mass spectra recorded directly from the two spots on a TLC slide of the chlorination reaction mixture (Scheme 1). The spectra are pictured in the same order, top to bottom, as the TLC spots from which they were taken.

to as high as 500 °C using the normal DART ionization source. In such cases (MW > ca. 600 amu), glass-backed TLC slides must be used, because the plastic backing will melt at temperatures exceeding 250 °C.[10]

The history of mass spectrometric analysis directly from TLC spots goes back more than two decades,[11] and the level of activity has risen considerably in recent years;[12,13] however, none of this work has been published in journals that are routinely read by synthetic organic chemists. The purpose of our report, therefore, is to bring this powerful new technology to the attention of the synthetic organic chemistry community. TLC−MALDI mass spectrometry has previously been demonstrated, but it is less convenient than

(7) For compounds that can be visualized by exposure to iodine vapors, we have found that the stained spots still yield good DART mass spectra. Slightly cleaner mass spectra are obtained by cutting the TLC slide first, staining only one half to locate the spots, and then using the other half for the mass spectrometric analysis. Phenolphthalein and acetaminophen can be visualized with iodine, but cinnamaldehyde and caffeine cannot.

(8) The TLC slides should be handled with tweezers to avoid introducing biological contaminants from the fingers of the operator. CAUTION: The thin beam of ionized gases that travels from the ion generator to the orifice on the mass spectrometer is hot, and fingers should be kept out of it.

(9) Kodomari, M.; Satoh, H.; Yoshitomi, S. *J. Org. Chem.* **1988**, *53*, 2093–2094.

(10) With glass TLC slides, the sample can be applied to the origin as a thin band, rather than as a single spot. It is then easier to cut through the middle of each eluted band. Alternatively, a single spot can be applied at the edge of the slide; however, elution behavior at the edge is often less uniform than at the center of the slide.

(11) For an early example, see: (a) Kushi, Y.; Handa, S. *J. Biochem.* **1985**, *98*, 265–8.

the open atmosphere technique described here, and it ordinarily requires trained staff personnel to perform the analyses. A major attraction of the DART-TOF mass spectrometer is the simplicity of its operation, which makes it accessible as a walk-up instrument (like NMR) to large numbers of users. It can be used, of course, in low-resolution mode if HRMS data are not needed.

In conclusion, we have found TLC−HRMS to be an effective method for the direct analysis of a wide variety of organic compounds that can be separated by thin layer chromatography. With the introduction of the DART ionization source, mass spectrometry is destined to become an essential everyday tool for the expeditious identification of components in crude organic reaction mixtures, not only at the completion of reactions, but even during the course of reactions, as new compounds are being formed.

Acknowledgment. We thank the National Science Foundation for financial support of this work through Grant No. 0414066 and for funds to purchase the mass spectrometer through Grant No. 0619576. Financial support from the Department of Energy through Grant No. 93ER14359 is also gratefully acknowledged.

OL8012759

(12) Recent TLC-MALDI: (a) Distler, U.; Huelsewig, M.; Souady, J.; Dreisewerd, K.; Haier, J.; Senninger, N.; Friedrich, A. W.; Karch, H.; Hillenkamp, F.; Berkenkamp, S.; Peter-Katalinic, J.; Muething, J. *Anal. Chem.* **2008**, *80*, 1835–1846. (b) Rohlfing, A.; Muething, J.; Pohlentz, G.; Distler, U.; Peter-Katalinic, J.; Berkenkamp, S.; Dreisewerd, K. *Anal. Chem.* **2007**, *79*, 5793–5808. (c) Fuchs, B.; Schiller, J.; Suess, R.; Schuerenberg, M.; Suckau, D. *Anal. Bioanal. Chem.* **2007**, *389*, 827–834. (d) Parent, A. A.; Anderson, T. M.; Michaelis, D. J.; Jiang, G.; Savage, P. B.; Linford, M. R. *Appl. Surf. Sci.* **2006**, *252*, 6746–6749. (e) Nakamura, K.; Suzuki, Y.; Goto-Inoue, N.; Yoshida-Noro, C.; Suzuki, A. *Anal. Chem.* **2006**, *78*, 5736–5743. (f) Dreisewerd, K.; Koelbl, S.; Peter-Katalinic, J.; Berkenkamp, S.; Pohlentz, G. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 139–150. (g) Dreisewerd, K.; Muething, J.; Rohlfing, A.; Meisen, I.; Vukelic, Z.; Peter-Katalinic, J.; Hillenkamp, F.; Berkenkamp, S. *Anal. Chem.* **2005**, *77*, 4098–4107.

(13) Other recent TLC−MS methods: (a) Haddad, R.; Milagre, H. M. S.; Catharino, R. R.; Eberlin, M. N *Anal. Chem.* **2008**, *80*, 2744–2750. (b) Van Berkel, G. J.; Tomkins, B. A.; Kertesz, V. *Anal. Chem.* **2007**, *79*, 2778–2789. (c) Morlock, G.; Ueda, Y. *J. Chromatogr., A* **2007**, *1143*, 243–251. (d) Lin, S.-Y.; Huang, M.-Z.; Chang, H.-C.; Shiea, J. *Anal. Chem.* **2007**, *79*, 8789–8795. (e) Van Berkel, G. J.; Kertesz, V. *Anal. Chem.* **2006**, *78*, 4938–4944.