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Thin-Layer Chromatography/Mass Spectrometry Analysis of Sample Mixtures Using a Compact Mass Spectrometer

Thin-layer chromatography (TLC) is a planar chromatographic technique used to separate the components of a mixture by employing a thin stationary phase supported by an inert backing.¹ It can be performed on the analytical scale to monitor the progress of a reaction, or on the preparative scale to purify small amounts of a compound. The technique is widely used because of its low cost, simplicity, relatively good sensitivity and speed of separation. Often, TLC spots are observed by UV or fluorescence light or by the use of chromogenic sprays that enhance detection, but these simple techniques do not provide meaningful information about the actual chemical compound within the spot. The analytical benefits of mass spectrometry could be realized if the composition of the TLC spots were analyzed by MS.

There have been a few reports on thin layer chromatography/mass spectrometry (TLC/MS)^{2,3} and thin layer chromatography/tandem mass spectrometry (TLC/MS/MS).^{4,5} The merits of TLC/MS (i.e., without MS/MS or high-resolution mass measurement) include lower-cost mass spectrometers, ease of operation and molecular weight (MW) information when applicable. TLC/MS/MS offers additional benefits, but requires more expensive instrumentation and more challenging operational requirements.

This article describes the direct coupling of TLC plates or other planar surfaces to a compact, single-quadrupole mass spectrometer that can provide routine electrospray ionization (ESI) or atmospheric pressure chemical ionization



Figure 1 – TLC/MS system used in this work. On the left is the compact mass spectrometer with the PlateExpress extraction device and on the right is the isocratic pump (Advion, Inc., Ithaca, N.Y.). Individual TLC spots were extracted via an extraction solvent delivered from the isocratic pump shown underneath the PlateExpress. The extract from the TLC spot was delivered directly to the ESI or APCI source of the mass spectrometer, which provides a mass spectrum of the TLC spot. Shown here is the Advion expression CMS-L.

(APCI) in both the positive and negative ionization modes. Information on molecular weight and fragmentation is made available using this process. Three application examples will be presented. *Figure 1* shows the system used in this work.

Experimental

Thin-layer chromatography

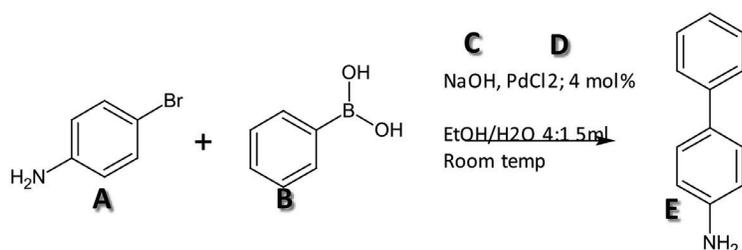
Silica gel 60 F₂₅₄ on aluminum plates (20 × 20 cm) were reduced in size to 10 × 10 cm (EMD/Merck KGaA, Darmstadt, Germany). The plates were placed in a 100 °C oven for 10 minutes prior to use to displace moisture.

Suzuki reaction

A representative Suzuki reaction and the experimental conditions used are shown in *Figure 2*. A 2- μ L aliquot of the crude reaction mixture from the stirred round-bottom flask was spotted onto the TLC plate at a position 1 cm above the bottom of the plate. Thirty milliliters of benzene was added to the developing chamber of a TLC plate at a depth of 0.5 cm. The spotted TLC plate was placed into the developing chamber and sealed with the glass lid. Separation was concluded when the development solvent front reached a position 1 cm from the top of the TLC plate. The TLC plate was then transferred to an oven and allowed to dry at 80 °C for 5 min. The separated “spots” were observed on the TLC plate under UV light at 254 nm.

Sudan dyes

Sudan I, Sudan II, Sudan III, Sudan IV, Sudan Orange G, Sudan Red 7B, Sudan Red B and all solvents used were purchased from Sigma-Aldrich (St. Louis, Mo.). Stock solutions at 1 mg/mL were prepared in a solvent mixture of MeOH/CHCl₃, 50/50 v/v. Chili powder was purchased from a local food store. Homogenized chili powder (2.5 g) was extracted with 25 mL of acetonitrile



	Reactants and Catalyst				Product
	A	B	C	D	E
compound	4-bromoaniline	Phenyl Boronic acid	Sodium hydroxide	Palladium chloride	4-aminobiphenyl
Chemical Formula	C ₆ H ₆ BrN	C ₆ H ₇ BO ₂	NaOH	PdCl ₂	C ₁₂ H ₁₁ N
MW	171	122	40	176	169
moles	0.005	0.005	0.0065	0.005	0.005
mg	855	610	260	35	845

Figure 2 – Chemical reaction for the Suzuki reaction described in the text. Reactants A and B were mixed at equimolar quantities in a round-bottom reaction flask and stirred at room temperature. Two-microliter aliquots were taken from the flask and spotted onto a TLC silica gel 60 F₂₅₄ plate (10 × 10 cm) at the times indicated.

by stirring for 15 minutes, followed by filtration through a 0.4- μ m filter. A dilution series of Sudan dye solutions was prepared at 0, 0.5, 5, 10, 20, 50 and 100 ng/ μ L and then fortified into the chili powder extract. The TLC developing solvent was ethyl acetate and hexane (15/85, v/v). For each concentration, 1 μ L of sample was spotted onto the TLC plate. The TLC spot elution solvent (0.1% formic acid in 50/50 MeOH/ACN) was delivered via an Advion LC isocratic pump at a flow rate of 250 μ L/min. Mass spectra were acquired in positive ion APCI mode.

Undergraduate student experiment: TLC/MS determination of caffeine in coffee

Freshly prepared, cooled coffee (5 μ L) was spotted onto the above-described TLC plate alongside 25 ng of standard caffeine. Ethyl acetate/hexane/acetic acid (20/10/0.5, v/v/v) was the developing solvent, and the developed plate was observed under UV at 254 nm. Full-scan APCI TLC/MS acquisition was used to analyze the caffeine spots (Figure 3).

Mass spectrometry

TLC/MS analyses were performed using the Plate Express extraction device coupled online to the expression CMS-L single quadrupole MS. This device incorporates an extraction head that forms a leak-tight seal on the surface of the TLC plate, which allows an extraction solvent to be delivered onto the TLC spot followed by a direct coupling to the inlet of the compact API mass spectrometer. The TLC plate extraction solvent/spray solvent was 0.1% formic acid in methanol or acetonitrile for the elution of the analytes from the TLC plate. Positive APCI MS analyses with full-scan or selected ion monitoring (SIM) mass spectral acquisition were as described.

Results and discussion

Synthetic reaction monitoring

The Suzuki reaction shown in Figure 2 is an organic reaction that is classified as a coupling reaction where the coupling partners are a boronic acid with a halide catalyzed by a palladium (0) complex.⁶ It is useful for the synthetic

chemist to know when the reaction is finished and that the expected product has been obtained. TLC screening of organic reaction media is routine for monitoring the progress of the reaction. In addition, whereas UV lamps and chromogenic sprays provide little information on the new spot, TLC/MS monitoring is able to provide the molecular weight and possibly fragmentation information observed in the TLC analysis of the crude reaction mixture.

Figure 4 shows the positive ion APCI extracted ion current (XIC) for the product and reactant of the Suzuki reaction depicted in Figure 2 along with the corresponding full-scan mass spectra for each. The reactant (4-bromoaniline) and the Suzuki reaction product (4-aminobiphenyl) are detected by their protonated molecules at m/z 172.0 (⁷⁹Br) and 174.0(⁸¹Br) and 170.1, respectively, at different time points from 0 to 180 minutes. Figure 4a shows the increase of the product with time via its XIC for m/z 170.1. The mass spectrum shown in Figure 4b was obtained from the product at the 120-minute time point. The additional mass spectral peak at m/z 202.1 in Figure 4b is consistent with the proton-bound adduct of methanol with the product. In Figure 4c, the XIC of the Suzuki reactant compound (protonated 4-bromoaniline) at m/z 171.9 (⁷⁹Br) is shown to decrease steadily through the time course of the reaction as it is consumed in the process. Figure 4d depicts a mass spectrum of the protonated Suzuki reactant 4-bromoaniline, showing the expected bromine doublet of the protonated molecule ions at m/z 171.9 and 173.9. The ability to monitor the course of an organic synthesis reaction can be very helpful to medicinal chemists or in synthetic reaction monitoring applications.

Sudan dyes in chili powder

Sudan dyes are synthetic azo-dyes that are widely used to provide color in plastics, leather, fabrics, oil, waxes, etc. They are classified as Group 3 carcinogens by the International Agency for Research on Cancer (IARC) and are banned as food additives worldwide.⁷ The application described here demonstrates the ability to easily detect the presence of seven Sudan dyes fortified into chili powder, separated by TLC and analyzed with online detection by mass spectrometry.

TLC/MS analysis of the original chili powder extract did not show the presence of the seven Sudan dyes studied (data not shown). Positive ion SIM TLC/MS analysis of the chili powder extract fortified with 0.5 ng for each dye applied to the TLC plate is shown in Figure 5. The TLC spot at a retention factor (Rf) of approximately 0.75 reveals ions at m/z 380.1, 249.1

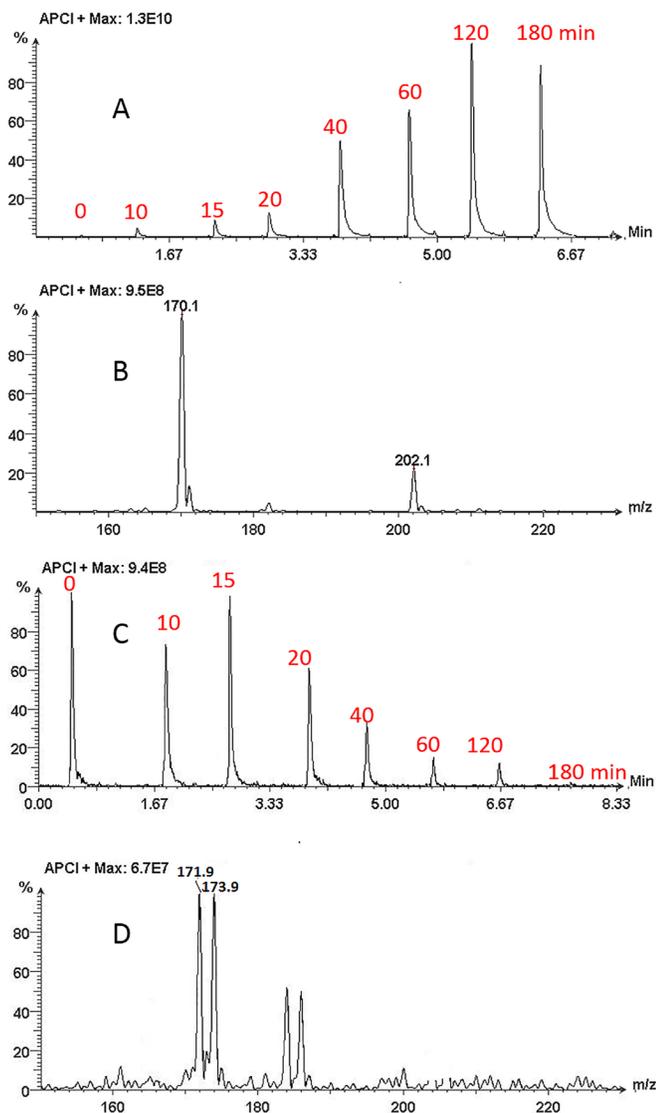


Figure 4 – The reactant (4-bromoaniline) and Suzuki reaction product (4-aminobiphenyl) were detected by their protonated molecules at m/z 171.9 and 170.1, respectively. a) Extracted ion current of the Suzuki reaction product (protonated 4-aminobiphenyl) at m/z 170.1 at different time points from 0 to 180 minutes. b) Mass spectrum of the product at m/z 170.1 at 120 minutes. c) Extracted ion current of the Suzuki reactant compound (protonated 4-bromoaniline) at m/z 171.9 taken at regular time intervals from 0 to 180 minutes. d) Mass spectrum of the protonated Suzuki reactant compound, 4-bromoaniline, showing the protonated molecule ions at m/z 171.9 and 173.9.

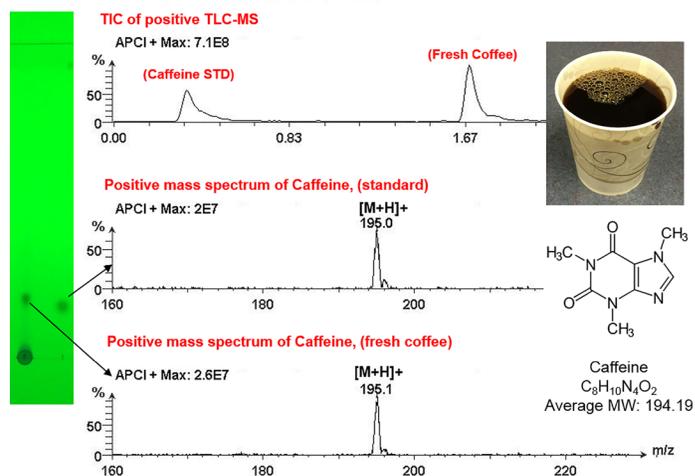


Figure 3 – Positive ion APCI TLC/MS of coffee spotted and developed on the TLC plate. The standard spot represents 25 ng of caffeine spotted onto the plate.

and 381.1. These protonated molecules are consistent with Sudan Red 7B, Sudan I and Sudan IV, respectively, which are not well separated on the TLC plate. However, their respective protonated molecules determined by TLC/MS makes it easy to distinguish these unresolved Sudan dyes.

Undergraduate and graduate student TLC/MS training experiments

Undergraduate chemistry laboratory training often includes TLC analysis of familiar samples such as coffee, soda and over-the-counter medicines. Although these experiments are interesting and relevant, students rarely have the opportunity to get hands-on experience with MS. The single-quadrupole mass spectrometer described here is well-suited for use in an undergraduate or graduate chemistry laboratory. The added complexities of LC/MS are precluded because the chromatography occurs on the TLC plate, which is easy to accomplish.

After cooling, 5 μ L of fresh coffee was added to the TLC plate alongside 25 ng of standard caffeine. Figure 3 shows the corresponding TLC spots at a Rf of approximately 0.2 after development of the TLC plate. The upper panel in Figure 3 is the total ion current (TIC) for full-scan positive ion APCI TLC/MS acquisition of the TLC spots for the caffeine standard and the coffee sample, respectively. Although these spots have slightly different Rf values, as shown in the left-hand panel, MS detection provides tentative confirmation of caffeine in the coffee via the respective mass spectra. The center panel shows the mass spectrum for the standard caffeine extracted from the TLC plate with its expected protonated molecule at m/z 195.1, while the lower panel displays the mass spectrum for caffeine from the coffee sample extracted from the TLC spot at Rf. 0.2. These mass spectra are identical, which supports the identification of caffeine in the coffee sample.

Conclusion

TLC/MS analysis of individual spots on a TLC plate can provide useful information on the chemical composition of a TLC spot. The

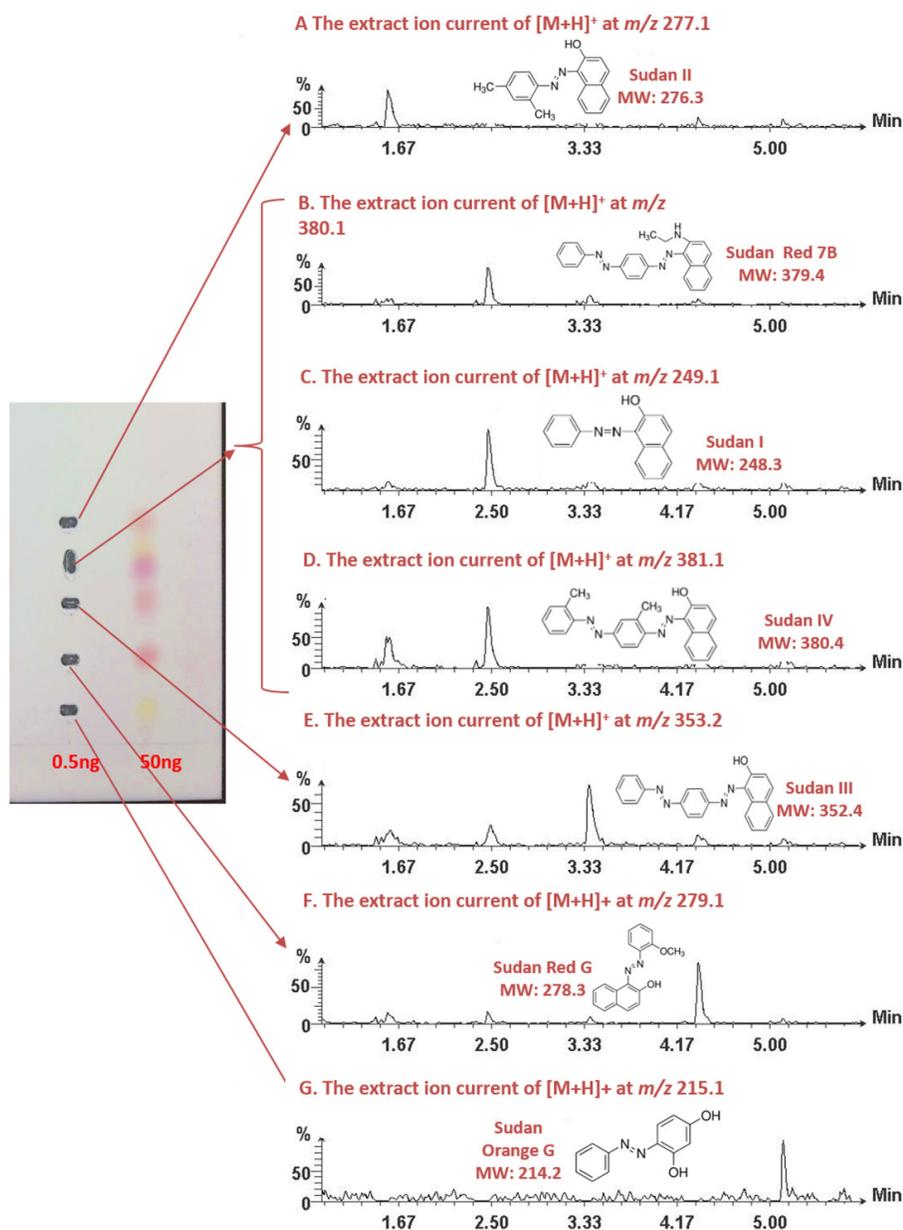


Figure 5 – SIM TLC/MS analysis of seven Sudan dyes (1 μ L containing 0.5 ng applied to the TLC plate) in chili powder extract. The selected ion current profiles for the seven Sudan dyes are shown in: a) Sudan II at m/z 277.1, b) Sudan Red 7B at m/z 380.1, c) Sudan I at m/z 249.1, d) Sudan IV at m/z 381.1, e) Sudan III at m/z 353.2, f) Sudan Red G at m/z 279.1 and g) Sudan Orange G at m/z 215.1.

technique is applicable for the real-time monitoring of synthetic reactions, detection of potential carcinogens in food and practical training of undergraduate and graduate chemistry students.

References

1. Sherma, J. Planar chromatography. *Anal. Chem.* **2010**, 82(12), 4895–910.
2. Cheng, S.C.; Huang, M.Z. et al. Thin layer chromatography/mass spectrometry. *J. Chromatogr. A* **2011**, 1218(19), 2700–11.
3. Van Berkel, G.J.; Tomkins, B.A. et al. Thin-layer chromatography/desorption electrospray ionization mass spectrometry: investigation of goldenseal alkaloids. *Anal. Chem.* **2007**, 79(7), 2778–9.
4. Henion, J.; Maylin, G.A. et al. Determination of drugs in biological samples by thin-layer chromatography-tandem mass spectrometry. *J. Chromatogr.* **1983**, 271(1), 107–24.
5. Van Berkel, G.J.; Ford, M.J. et al. Thin-layer chromatography and mass spectrometry coupled using desorption electrospray ionization. *Anal. Chem.* **2005**, 1207–15.
6. Miyaura, N.; Yamada, K. et al. A new stereospecific cross-coupling by the palladium-catalyzed reaction of 1-alkenylboranes with 1-alkenyl or 1-alkynyl halides. *Tet. Lett.* **1979**, 20(36), 3437–40.
7. Stiborová, M.; Martínek, V. et al., Sudan I is a potential carcinogen for humans: evidence for its metabolic activation and detoxication by human recombinant cytochrome P450 1A1 and liver microsomes. *Cancer Res.* **2002**, 62, 5678–84.

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