TLC and APCI-MS Coupled Using a Surface Sampling Heated Nebulizer Probe

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OVERVIEW

- **Purpose:** Design and construct a self-aspirating heated nebulizer probe for direct analysis of surfaces and liquids by atmospheric pressure chemical ionization.
- **Method:** Modification of an LCQ Deca APCI source (Thermo Finnigan, San Jose, CA)
- **Results:** Successful analysis of dyes separated on a thin-layer chromatography plate, analysis of pharmaceutical tablets, living tissue, and sampling liquid samples from a 384-well microtiter plate.

INTRODUCTION

- We recently reported on a coupled thin-layer chromatography/electrospray mass spectrometry (TLC/ES-MS) system that makes use of a combined surface sampling probe/ES emitter as the coupling interface.[1,2,3]
  - Takes advantage of formation of liquid micro-junction between the sampling probe and TLC plate and a self-aspirating ES emitter for the direct read of developed TLC plates by ES/MS.
  - Provides a complement to the recently popular matrix-assisted laser desorption/ionization (MALDI) approaches for coupling TLC and MS.[4,5]
- Similar self-aspirating ES was first described by Wachs and Henion[6]
  - Developed a micro ion spray device for coupling chip based separation technologies and a related multi-well plate and surface sampling with ES-MS.
  - Analyte elution from a solid-phase sorbent extraction card using this device.[7]
- Other examples of direct sampling of liquids with ES-MS include Karger and coworkers[8]
  - Developed an infusion system using a subatmospheric ES interface for the direct sampling from microtiter plates without the need of a separate solvent delivery system.
- ES is not a universal ionization source.[9] The ability to use other spray ionization methods[10] employing the basic self-aspirating ion source approach would expand the range of compounds amenable to analysis and expand the versatility of direct sampling.
- One of the more important of these ionization methods is atmospheric pressure chemical ionization (APCI). In comparison to ES[9], APCI is generally more suitable for less polar molecules of lower molecular mass (< ca. 2000 Da).[11] In our laboratory we have successfully coupled rotation planar chromatography on-line with the self-aspiration capability of the APCI source.[12]
- In this presentation, we report on a straightforward modification of the heated nebulizer portion of the APCI source from a quadrupole ion trap mass spectrometer that transforms it into a self-aspirating sampling probe for the direct analysis of analytes on surfaces and in liquid samples.

SURFACE SAMPLING PROBE

Self-Aspirating APCI Source

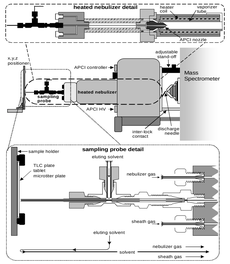


Figure 1 – Schematic illustration of the modified LCQ Deca APCI source (Thermo Finnigan)

- 18.5 cm long stainless steel sampling/spray capillary (31 gauge, 127 mm-i.d., 254 mm-o.d.) replaced the fused silica transfer capillary.
- A concentric 24 gauge stainless steel tube (305 mm-i.d., 559 mm-o.d.) was slipped over the end of the sampling tube, held in place with a 10-32 nut and ferrule and a 1/16"-o.d. Teflon sleeve.
- Inner sampling tube was positioned ca. 50-100 mm inside the outer capillary.
- Sample holder and stage described previously[2], controlled by an MS2000 x,y,z robotic platform (Applied Scientific Instrumentation Inc.)

EXPERIMENTAL

Mass Spectrometer Conditions:

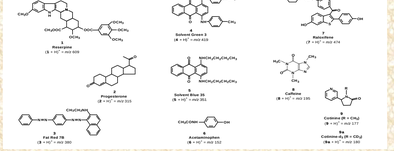
vaporizer temperature	400 °C	capillary voltage	5 V
capillary temperature	150 °C	tube lens offset	-10 V
discharge current	2 µA	1st multipole offset	-8.25 V
sheath gas flow	40 arb units	2nd multipole offset	-11 V
auxiliary gas flow	10 arb units	lens voltage	-20 V
rf amplitude	400 V_{pp}	entrance lens	-64 V

MS/MS experiments carried out at 35% normalized collision energy with a 30 ms activation time

TLC Experimental

- 20 x 10 cm HPTLC hydrophobic Merck RP C18 plate with 2.5 x 20 cm pre-concentration zone, cut into 5 cm (w) x 10 cm (l) sections prior to use to fit sample holder.
- Samples spotted onto plates in 1.0 µL aliquots.
- Developing solvent was isopropanol, plates dried (110 °C) for 10 minutes.
- Color photograph of the developed plate taken with a Coolpix 990 digital camera using white light illumination.

Chemicals



RESULTS AND DISCUSSIONS

Operation as a self-aspirating APCI source

- Self-aspiration flow rate depends upon the sheath or nebulizing gas flow rate and on the composition of the solvent being aspirated.
- Solvent flow as high as 75 mL/min with methanol and 60 mL/min with acetonitrile.
- Co-axial flow of nebulizing gas in the probe creates a local vacuum at the end of the inner capillary spray tip.
- Vacuum draws liquid from the sampling end of the probe that enters from the syringe driver through the capillary annulus or from the liquid in a well being sampled through the inner capillary and then sprayed into the heated region of the probe.
- For sampling from surfaces, parameters adjusted so the eluting solvent flow rate and self-aspiration flow rate were balanced.

APCI in self-aspiration mode

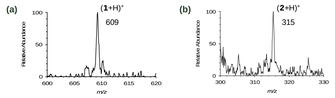
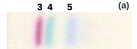


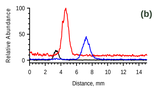
Figure 2 – Single, full scan mass spectra from the self-aspiration of (a) a 25 fmol/µL solution of reserpine (1), prepared in 50/50 (v/v) acetonitrile/water with 0.1% acetic acid at a flow rate of 30 µL/min and (b) a 100 fmol/µL solution of progesterone (2), (same solvent as for 1) at flow of 25 µL/min, illustrate the spectral intensity generated for these compounds at solution concentrations near their detection limits.

Detection limits were determined by linear regression analysis (Microsoft Excel) on calibration curves (peak intensity of *m/z* 609 vs. reserpine concentration and *m/z* 315 vs. progesterone concentration) using the upper 95% confidence limit of the y-intercept. The detection limit for reserpine was 6.5 fmole/µL and 51 fmole/µL for progesterone.

Surface Sampling of TLC Plate

- Eluting solvent 50/50 tetrahydrofuran with 0.1% acetic acid
- TLC plate scanned relative to the probe along the development lane at 44 µm/s





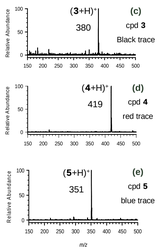
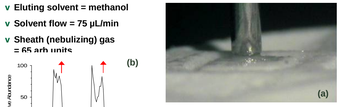


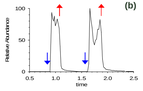
Figure 3 – (a) Photograph of RP C18 TLC plate development lane showing the separated components of a spotted mixture (50 ng each components) of fat red 7b (3, *m/z* 380), solvent green 3 (4, *m/z* 419) and solvent blue 35 (5, *m/z* 351). (b) Mass chromatograms for *m/z* 380 (black trace), *m/z* 419 (red trace), and *m/z* 351 (blue trace) normalized to the signal levels for *m/z* 419. The background subtracted, averaged mass spectra acquired over the peak in the chromatograms corresponding to the respective bands on the plate are (c) fat red 7b (3), (d) solvent green 3 (4), and (e) solvent blue 35 (5).

The self-aspirating APCI source can also be used to sample pharmaceutical tablets and other surfaces much like the other new surface sampling techniques such as desorption electrospray ionization (DESI)[13,14] and direct analysis in real time (DART)[15].

Surface Sampling of an Extra Strength Tylenol® tablet

- Eluting solvent = methanol
- Solvent flow = 75 µL/min
- Sheath (nebulizing) gas = 65 arb units





↓ indicates when probe forms liquid junction with surface

↑ indicates when probe removed from surface and liquid junction is broken

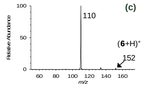
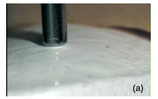
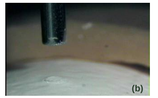


Figure 4 – (a) Photograph of the Extra Strength Tylenol® tablet being sampled by the APCI surface sampling probe.
(b) Chromatogram monitoring the product ion scan of *m/z* 152 showing sharp increase in signal when probe is lowered to surface (down arrow) and sharp decrease when probe is raised from surface (up arrow).
(c) Product ion spectrum showing ketene loss from the protonated molecule to yield the characteristic base peak for acetaminophen (6) at *m/z* 110[16].

Surface Sampling of an Evista® tablet





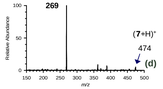
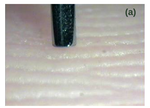
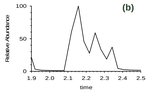


Figure 5 – (a) Photograph illustrating the sampling of an Evista® tablet with the APCI surface sampling probe.
(b) Photo showing the depression left where liquid junction was formed during sampling.
(c) Chromatogram showing gradual increase in signal for the product ion spectrum (*m/z* 150-1000) of *m/z* 474 (protonated raloxifene (7) molecule as tablet coating is dissolved.
(d) Averaged MS/MS spectrum for selected time period (t = 1.04 to 1.74 min in blue) showing a characteristic product ion at *m/z* 269. The more commonly monitored product ion with triple quadrupole instruments at *m/z* 112 was below the set scan range of the ion trap[17].

Surface Sampling from a Finger

- Eluting solvent: methanol
- Solvent flow = 75 µL/min





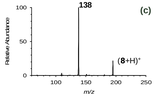
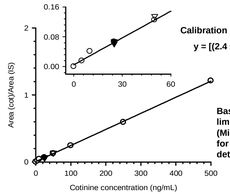


Figure 6 – (a) Photograph of the APCI probe sampling a finger that just handled a Vivarin® tablet.
(b) Chromatogram monitoring the product ion scan of *m/z* 195, protonated caffeine (8) molecule.
(c) Product ion spectrum of *m/z* 195 showing product ion at *m/z* 138.

Sampling Analyte Solutions from a Microtiter Plate

- 384-well microtiter plate (MJ Research, Inc., Watertown, MA) with cotinine (9) standards, each with 500 ng/mL cotinine-d_3 (9a) as an internal standard, affixed to sample holder.
- Wells sampled for 1 minute, probe flushed for 1 minute.
- Eluting solvent = acetonitrile
- Eluting solvent flow rate = 10 µL/min
- Total aspiration rate = 60 µL/min.
- MS/MS acquisition programmed to alternate between two selected reaction transitions, *m/z* 177 ® 146 for cotinine and *m/z* 180 ® 146 for cotinine-d_3.



Calibration data fit the model:

$$y = [(2.4 \pm 0.017)10\text{-}3]x + (5.0 \pm 3.4)10\text{-}3$$

Based on upper 95% confidence limit, using regression analysis (Microsoft Excel), a detection limit for cotinine of 31 fmole/µL was determined.

Figure 7 – Calibration curve generated from sampling wells with the APCI probe. Eluting solvent flow rate was set to allow washout between samples but minimize dilution of the samples. Inset shows the lower concentration region. (o) = cotinine standards (s) = 25 ng/mL quality control, (▼) = 50 ng/mL quality control. Two of the cotinine standards, 25 ng/mL (twice) and 50 ng/mL, were resampled and served as quality control points. Recoveries for these samples were 94.4% and 104.8% for the 25 ng/mL standard, and 109% for the 50 ng/mL standard.

CONCLUSION

- Successful fabrication and operation of a self-aspirating heated nebulizer probe for APCI MS.
- Demonstration of the APCI surface sampling probe for the direct analysis of
  - TLC plates
  - Pharmaceutical tablets
  - Living tissue, such as a finger
  - Liquid samples from a microtiter plate

REFERENCES

1. Van Berkel, G. J.; Sanchez, A. D.; Quirke, J. M. E. *Anal. Chem.* **2002**, *74*, 6216-6223.
2. Ford, M. J.; Van Berkel, G.J. *Rapid. Commun. Mass Spectrom.* **2004**, *18*, 1303-1309.
3. Ford, M. J.; Kertesz, V.; Van Berkel, G. J. J. *Mass Spectrom.*, submitted.
4. Gusev, A. L. *Fresenius J. Anal. Chem.* **2000**, *366*, 691-700.
5. Crecelius, A.; Clench, M. R.; Richards, D. S. *LCGC Europe*, **2003**, *16*, 225-229.
6. Wachs, T.; Henion; J. *Anal. Chem.* **2001**, *73*, 632-638.
7. Wachs, T.; Henion, J. *Anal. Chem.*, **2003**, *75*, 1769-1775.
8. Felten, C.; Foret, F.; Minarik, M.; Goetzinger, W.; Karger, B.L. *Anal. Chem.*, **2001**, *73*, 1449-1454.
9. Cech, N. B.; Enke, C. G. *Mass Spectrom Rev.* **2001**, *20*, 362-387.
10. Van Berkel, G. J. *Eur. J. Mass Spectrom.* **2003**, *9*, 539-562.
11. Bruins, A. P. *Mass Spectrom. Rev.* **1991**, *10*, 53-77.
12. Van Berkel, G.J.; Llave, J.J.; De Apadoca, M.F.; Ford, M.J. *Anal. Chem.*, **2004**, *76*, 479-482.
13. Takáts, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R.G. *Science*, **2004**, *306*, 471-473.
14. Van Berkel, G.J.; Ford, M.J.; Deibel, M.A. *Anal. Chem.*, **2005**, *77*, 1207-1215.
15. Cody, R.B.; Laramée, J.A.; Durst, H.D. *Anal. Chem*, **2005**, *77*, 2297-2302.
16. Celma, C.; Allué, J.A.; Pruñonosa, J.; Peraire, C.; Obach, R. *J. Chrom. A*, **2000**, *870*, 77-86.
17. Zweigenbaum, J.; Henion, J. *Anal. Chem.* **2000**, *72*, 2446-2454.

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