

Surface Analysis and Chemical Imaging with DESI: Technology-Related Challenges and Solutions

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OVERVIEW

- Goals:**
- Evaluate effects of surface sampling parameters/methods on DESI signal reproducibility for high-throughput applications
 - Study DESI parameters affecting chemical image quality
- Methods:**
- DESI signal reproducibility:
 - Spot sampling
 - Line scanning
 - Image quality
 - Surface-to-sampling capillary distance
 - Scanning mode (uni- and bidirectional)
 - Sub-plume size resolution
- Results:**
- Best reproducibility achieved for spot sampling if the sample spot is approached as quickly as possible
 - Good reproducibility can be obtained at very fast scan rates using line scanning
 - Changing sampling capillary-to-surface distance during imaging may result in signal loss and incorrect spatial assignment of the area sampled on the surface, but can be controlled through automation.
 - Imaging using unidirectional scanning provides more accurate signal-to-surface spatial location assignment over bidirectional scanning.
 - Sub-plume size vertical image resolution (ca. 50 μ m) is possible because of the size of the most efficient desorption/ionization region ("sweet spot").

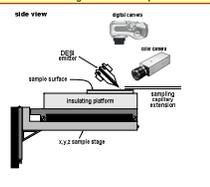
INTRODUCTION

- This research is focused on understanding and controlling technology-related parameters affecting DESI signal reproducibility for high-throughput analysis and affecting imaging quality using desorption electrospray ionization mass spectrometry [1-3].
- DESI-MS has been used before to demonstrate high-throughput monitoring of pharmaceutical samples [4] using a variable-speed moving belt providing rapid qualitative and semi-quantitative information on drug constituents in tablets.
- Recently, the technique was used to demonstrate imaging of analyte bands on TLC plates [5] and imaging endogenous compounds in rat brain tissue [6-7].
- To-date investigations have focused on the effect of spray parameters on the visible-LC spatial resolution/spot size [2,5-7].
- In this work we report on
- optimization of DESI signal reproducibility for spot sampling and line scanning
 - the effects of scanning direction (uni- or bidirectional scanning) and sampling capillary-to-surface distance on image quality
 - development of an image analysis automation concept and associated software to control the sampling capillary-to-surface distance during surface scans to achieve maximum DESI signal and to ensure accurate spatial assignment of the area sampled
 - sub-plume size vertical image resolution

EXPERIMENTAL

- Chemicals.** HPLC grade acetonitrile (ACN), methanol and isopropanol (IPA) were purchased from Burdick & Jackson (Muskegon, MI), methanamine 60 (1) and B (2) were from Eastman Kodak Company (Rochester, NY).
- Spot Sampling/Lane Scanning.** Square-shaped sample spots (3x3 mm in size with 5-mm spacing unless otherwise noted) were printed with an Epson Stylus Photo R340 printer on premium copy paper (Hammermill, Item # 04050, International Paper Company, Memphis, TN) using a 0.1 mm 100 μ m 210isopropanol solution as ink. MRM signal collected for 1 (m/z 443.3 \pm 415.3) used as sample analyte signal, while MRM signal collected for 2 (m/z 443.3 \pm 399.3) used as an internal standard. Results from analyses of 10 sample spots were used to calculate relative standard deviation (RSD).
- Lines were drawn on 75x25 mm glass microscope slides (Fisherbrand, Cat. No. 12-0442; Fisher Scientific, Pittsburgh, PA) and fully frosted glass microscope slides (Fisherbrand, Cat. No. 12-544-0CY, Fisher Scientific) using red permanent markers (Fine Sharpie (ser. no. 30000) and UltraFine Sharpie (ser. no. 37000), Sanford Corporation, Oak Brook, IL). The principal dye in the red ink was C, determined by its parent ion at m/z 443.3 and by product ion spectrum (not shown).**
- Imaging Primed Lines/Caps/Sharpies on Copy Paper.** Lines/caps/primed lines/shapes were printed on copy paper (Hammermill Great White Copy, Item # 80700) using an Epson Stylus Color 600 printer with its default 500x600 color cartridge and the distribution of the most intense *n*-mer of a polymer additive in the ink at m/z 493.3 was monitored. Both the full mass spectrum of the ink and the product ion spectrum of m/z 493.3 were recorded in positive ion mode (not shown).
- High-Resolution Imaging.** Visible and fluorescent images of printed grids on normal-phase TLC plates were acquired with a Zeiss Axiovert 2 FS plus fluorescence microscope (Carl Zeiss Microscopy, Jena, Germany).

Figure 1. DESI Setup



SPOT SAMPLING

Figure 2. DESI Signal Reproducibility as a Function of Analysis and Dwell Times

Figure 4. DESI Signal Reproducibility as a Function of Sampling Mode

Figure 3. DESI Signal Reproducibility as a Function of Surface Approach Speed

Figure 5. DESI Signal Reproducibility as a Function of Surface Scan Speed and Dwell Time

Figure 6. DESI Signal Reproducibility as a Function of Sample Spot Size and Spacing

Figure 7. Effect of Capillary-to-surface Distance (d_{cap}) on the Quality of the Chemical Image

Figure 8. Effect of Scanning Mode on Signal-to-surface Spatial Location Assignment

Figure 9. Effect of Scanning Mode on DESI Signal Abundance

CHEMICAL IMAGING

Figure 7. Effect of Capillary-to-surface Distance (d_{cap}) on the Quality of the Chemical Image

Figure 10. DESI Plume Map

Figure 11. High-resolution imaging with DESI

Figure 8. Effect of Scanning Mode on Signal-to-surface Spatial Location Assignment

Figure 9. Effect of Scanning Mode on DESI Signal Abundance

Figure 10. DESI Plume Map

Figure 11. High-resolution imaging with DESI

CONCLUSIONS

- Best DESI signal reproducibility is observed in spot sampling if
 - the sample spot is approached as quickly as possible AND the DESI plume leaves the sample spot as quickly as possible following analysis by a path that avoids efficient desorption of fresh analyte surface area.
- Good reproducibility can be obtained even at very fast surface scan speeds allowing high-throughput sample analysis using line scanning. Analysis time of ca. 0.5 s/sample was achieved.
- Non-optimal and/or changing sampling capillary-to-surface distance results in signal loss and incorrect spatial assignment of the area sampled.
- Image analysis automation concept and associated software (Hands-Free Surface Analysis) enables control and "hands-free" reoptimization of the sampling capillary-to-surface distance during surface scans to achieve maximum DESI signal and to ensure correct spatial assignment of the area sampled to its true surface location.
- Unidirectional scanning provides more accurate signal-to-surface spatial location assignment over bidirectional scanning.
- Vertical image resolution higher than expected based on the vertical plume size is possible because of the size of the most efficient desorption/ionization region ("sweet spot") size.

NEAR FUTURE

- Develop faster surface/analyte spot approaches to obtain
 - more reproducible results/lower RSDs
 - higher throughputs
- Study the effect of
 - analyte-surface interaction on reproducibility
 - surface parameters on reproducibility
 - roughness
 - spray-to-surface distance
 - divergence angle
- High-throughput studies with real-life samples
- High-resolution chemical imaging of tissues

REFERENCES

[1] Takka, Z. et al., *Science* 2004, 306, 417-472.
 [2] Takka, Z. et al., *J. Mass Spectrom.* 2004, 39, 1281-1295.
 [3] Cook, R. G. et al., *Science* 2006, 311, 1596-1597.
 [4] Chen, R. et al., *Anal. Chem.* 2005, 77, 8935-8937.
 [5] Van Berkel, G. J. et al., *Anal. Chem.* 2006, 78, 6938-6944.
 [6] Wilman, J. M. et al., *Angew. Chem. Int. Ed.* 2005, 44, 7188-7192.
 [7] He, R. et al., *Anal. Mass Spectrom.* 2007, 230, 5-15.

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