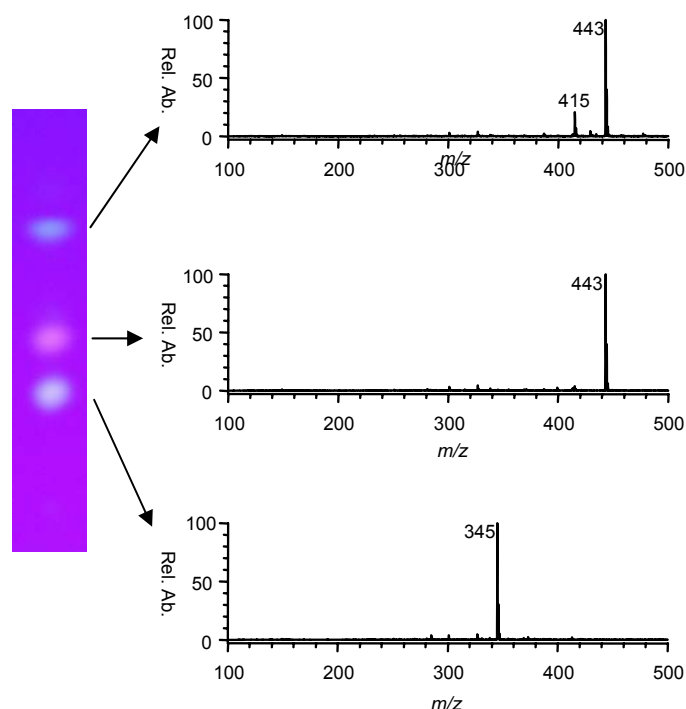


Advanced Mass Spectrometric Approaches for the Analysis of Analytes on Planar Separation Media

Vilmos Kertesz

**Organic and Biological Mass Spectrometry Group,
Chemical Sciences Division**

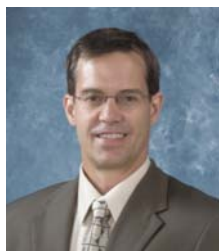


**Presented at the HPTLC
2008 Conference**

June 11-13, 2008

Acknowledgements

People



Gary Van Berkel



Michael Ford
(NextGen Sciences)



Sofie Pasilis



Bruce Tomkins

Collaboration - Instrumentation



Tom Covey
Brad Schneider



Julian Phillips



Rick King
Ken Koeplinger
Marissa Vavrek

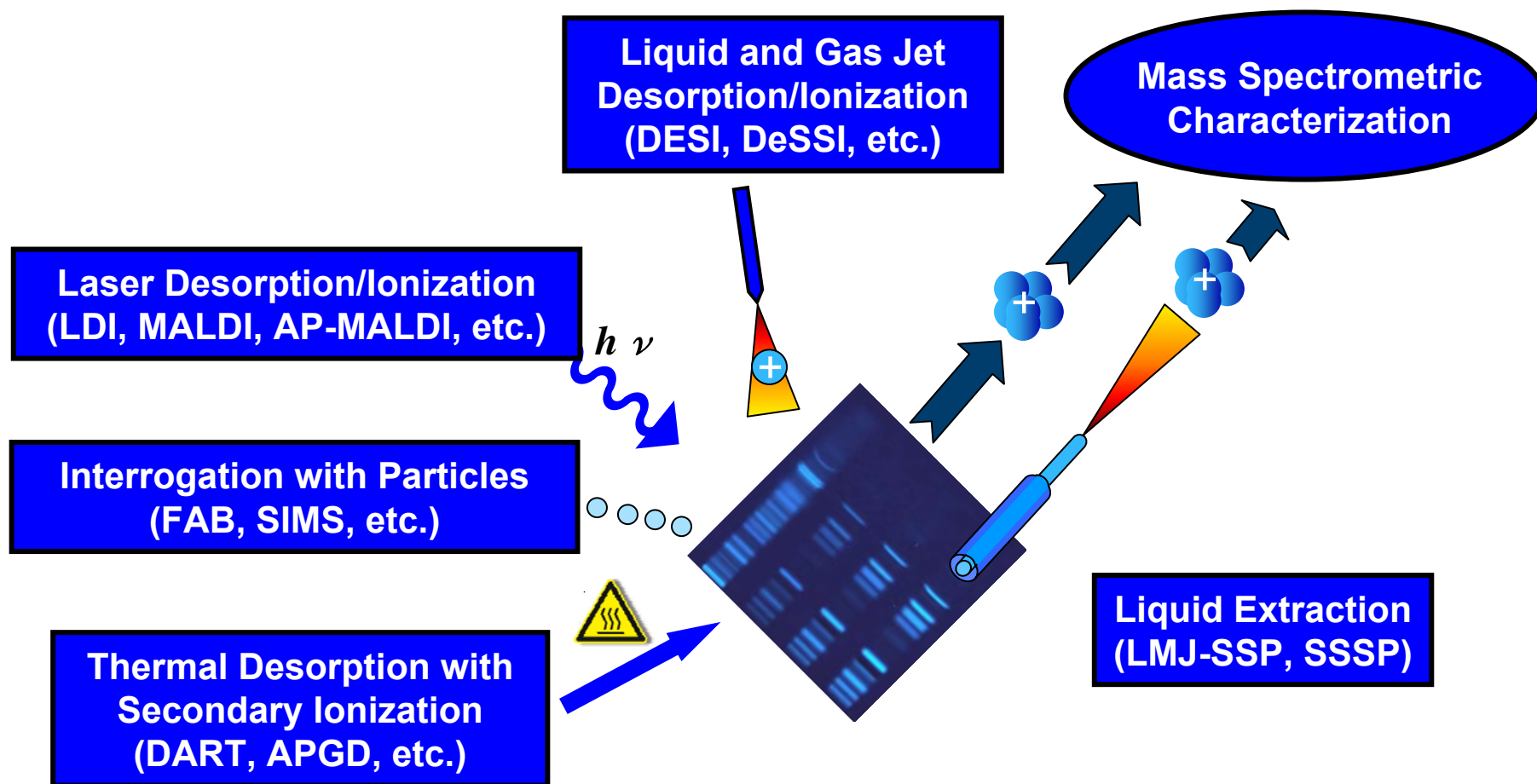


Michael Schulz
Susanne Schorcht
Dagmar Leiss

Funding



Atmospheric Pressure Surface Sampling/Ionization Methods for Mass Spectrometry



Liquid and Gas Jet Desorption/Ionization

Desorption Electrospray Ionization (DESI)

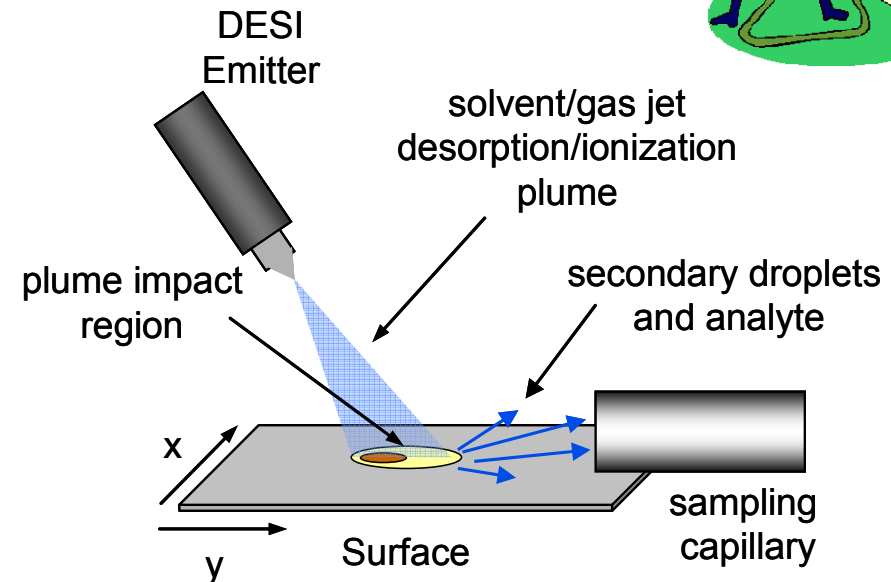
- DESI is a multiple-step process
 - liquid-solid extraction
 - transfer of extract into gas phase/ionization

Liquid-solid extraction

- surface/analyte characteristics
- DESI impact plume characteristics
- solvent, solvent flow rate, gas flow rate
- extraction time (surface scan rate when scanned)

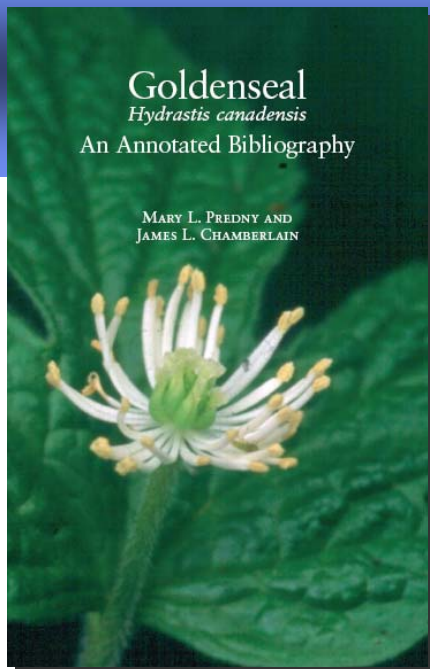
Transfer into gas phase/ionization

- droplet/ion transfer to gas phase
- droplet/ion transfer in the sampling capillary
- ion identity, charge state
- analyte modification (e.g., oxidation)



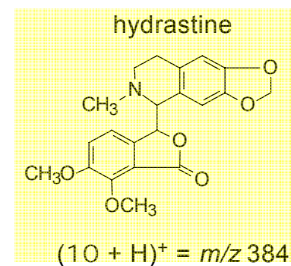
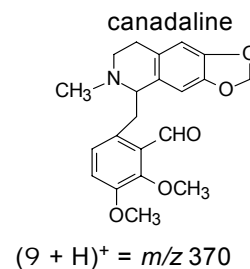
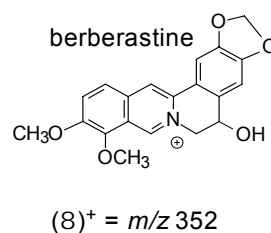
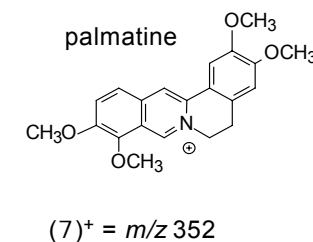
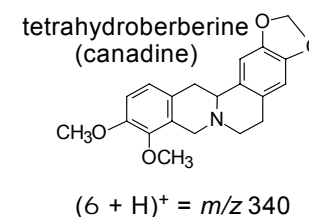
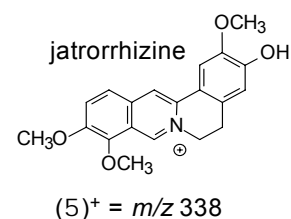
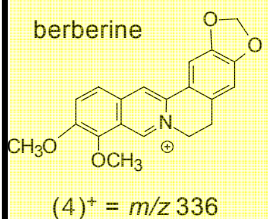
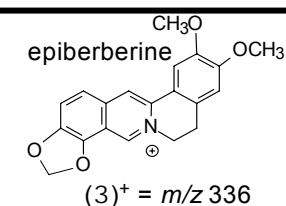
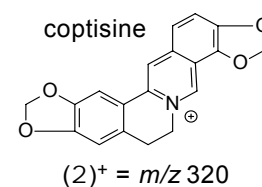
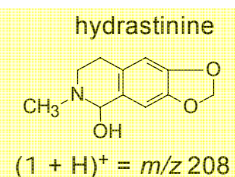
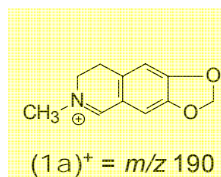
Desorption Sonic Spray Ionization (DeSSI)

Turn off the high voltage and turn up nebulizing gas velocity



TLC/DESI-MS: Goldenseal

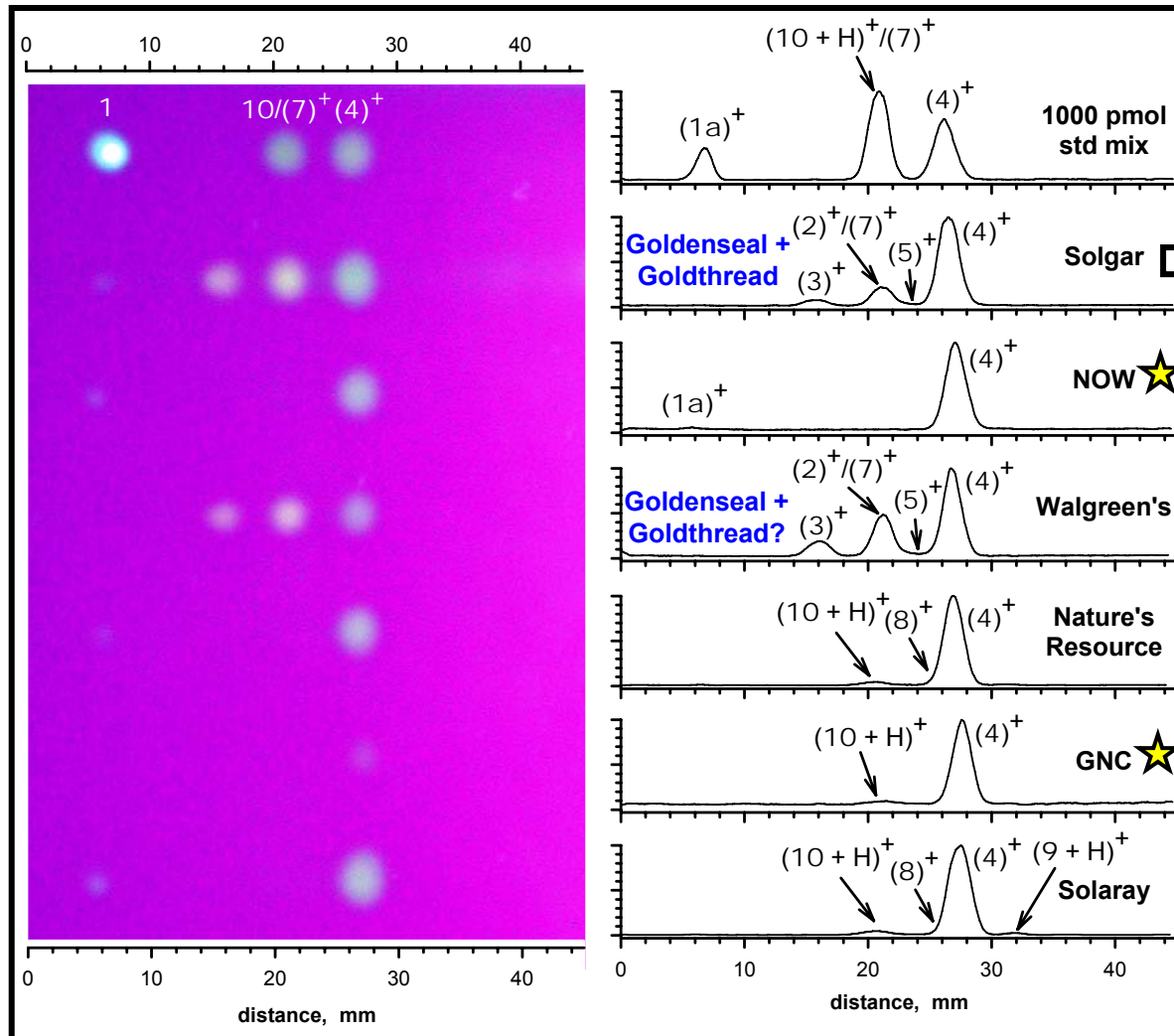
- A top-selling herbal product in USA
- Berberine, hydrastine, hydrastinine
- Substitute/admix with other alkaloid containing herbs (e.g., Goldthread)



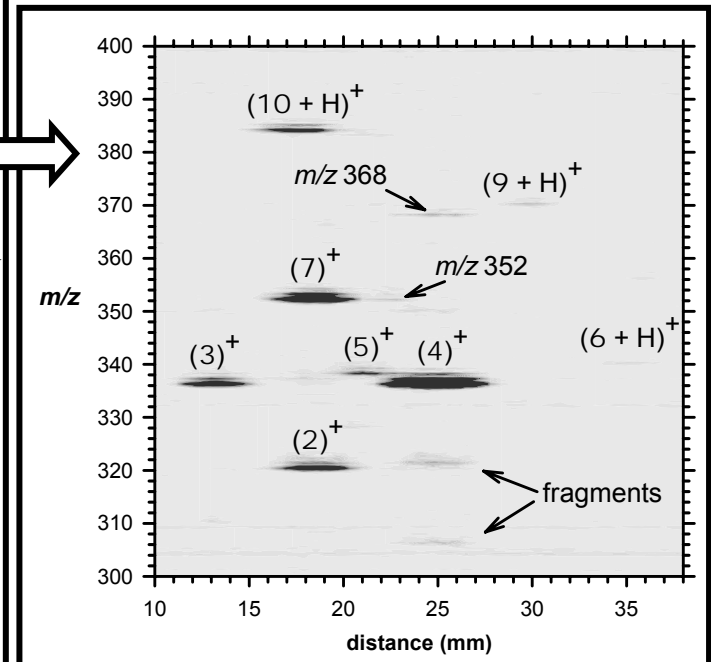
Van Berkel, et al., *Anal. Chem.* 79, 2778 -2789, 2007

TLC/DESI-MS: Goldenseal

Analysis of six commercial "Goldenseal" products



Solgar sample ion map



- Sample complexity revealed in ion map
- Considerable component overlap in separation lane
 - Detection and quantitation by simple optical techniques compromised

NP-glass back HPTLC plate
50/10/6/3 v/v/v/v ethyl acetate/methanol/formic acid/water

TLC/DESI-MS: Goldenseal

Quantification Results for Goldenseal Alkaloids in Two Commercially-Available Brands Determined Using TLC/DESI-MS and Fluorescence Spectroscopy, and Compared with Label Values

	Method of Quantitation	Calculated Mass of Alkaloid per Capsule, mg ¹		
		Berberine	Palmatine	Hydrastinine ²
Solgar	TLC/DESI-MS	16 ± 2.3; n = 4	2.2 ± 0.37; n = 4	<0.24
	Fluorescence	19 ± 0.86; n = 3	8.4 ± 0.47; n = 3	< 0.24
	Label Value ³	15		
Nature's Resource	TLC/DESI-MS	12 ± 0.91; n = 4	not detected	<0.24
	Fluorescence	14 ± 1.2; n = 3	not detected	< 0.24
	Label Value ⁴	13.4		

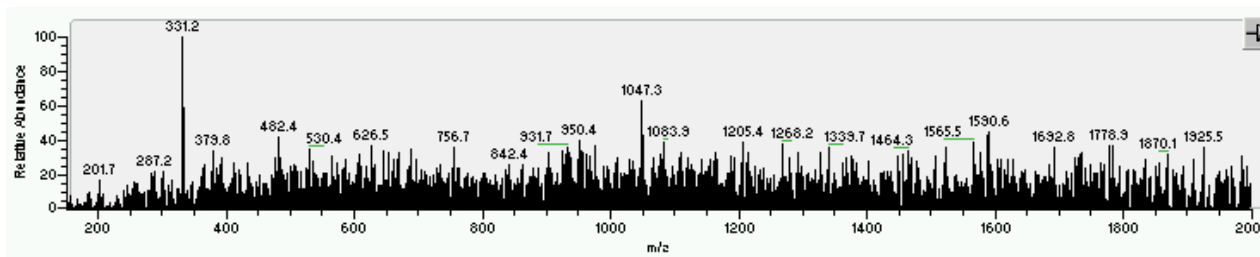
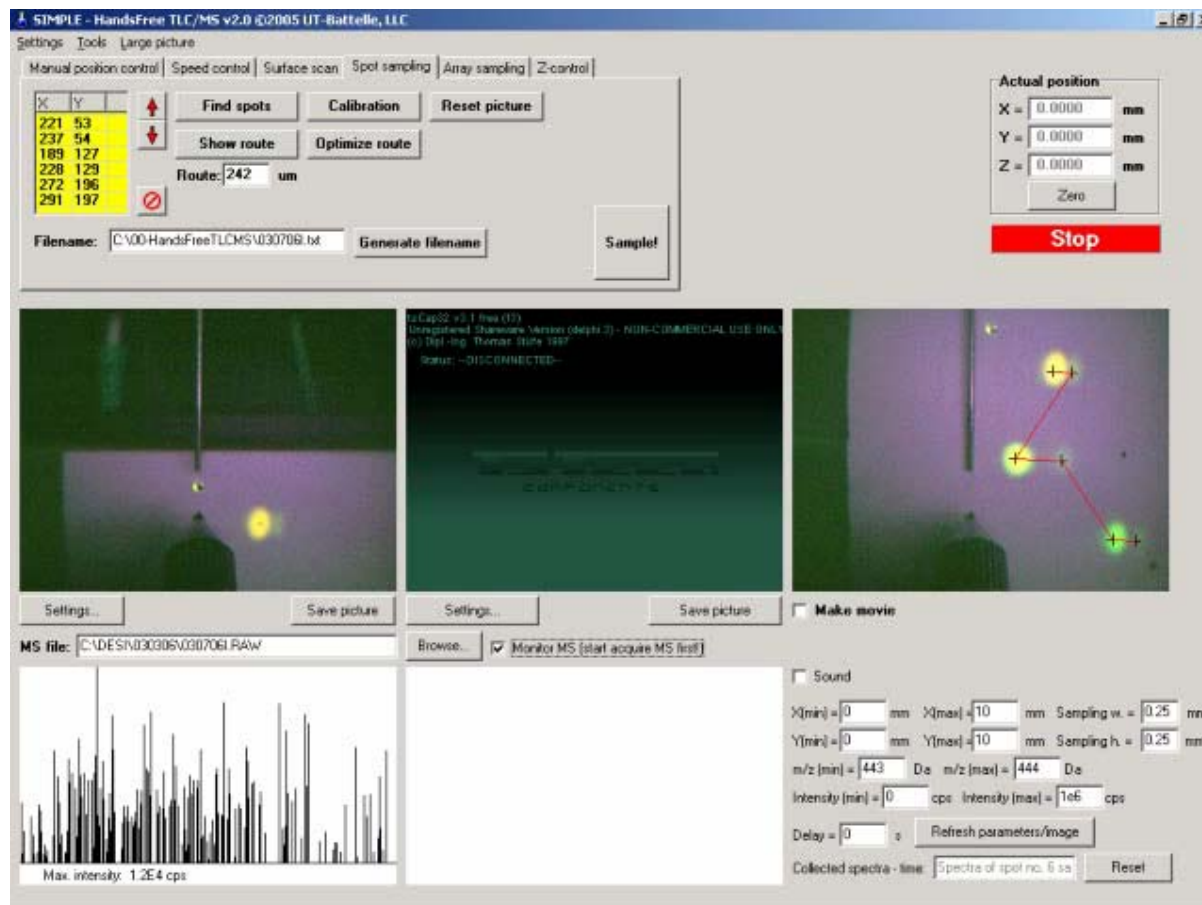
¹ Reporting convention is mean ± standard deviation based on “n” replicates.

² Hydrastinine was observed, but at a mass below its calculated detection limit of 0.24 mg/capsule for both DESI-MS and fluorescence.

³ Estimated total alkaloid content is 15 mg/capsule, based on the label values. This value has been assigned to berberine for comparison purposes.

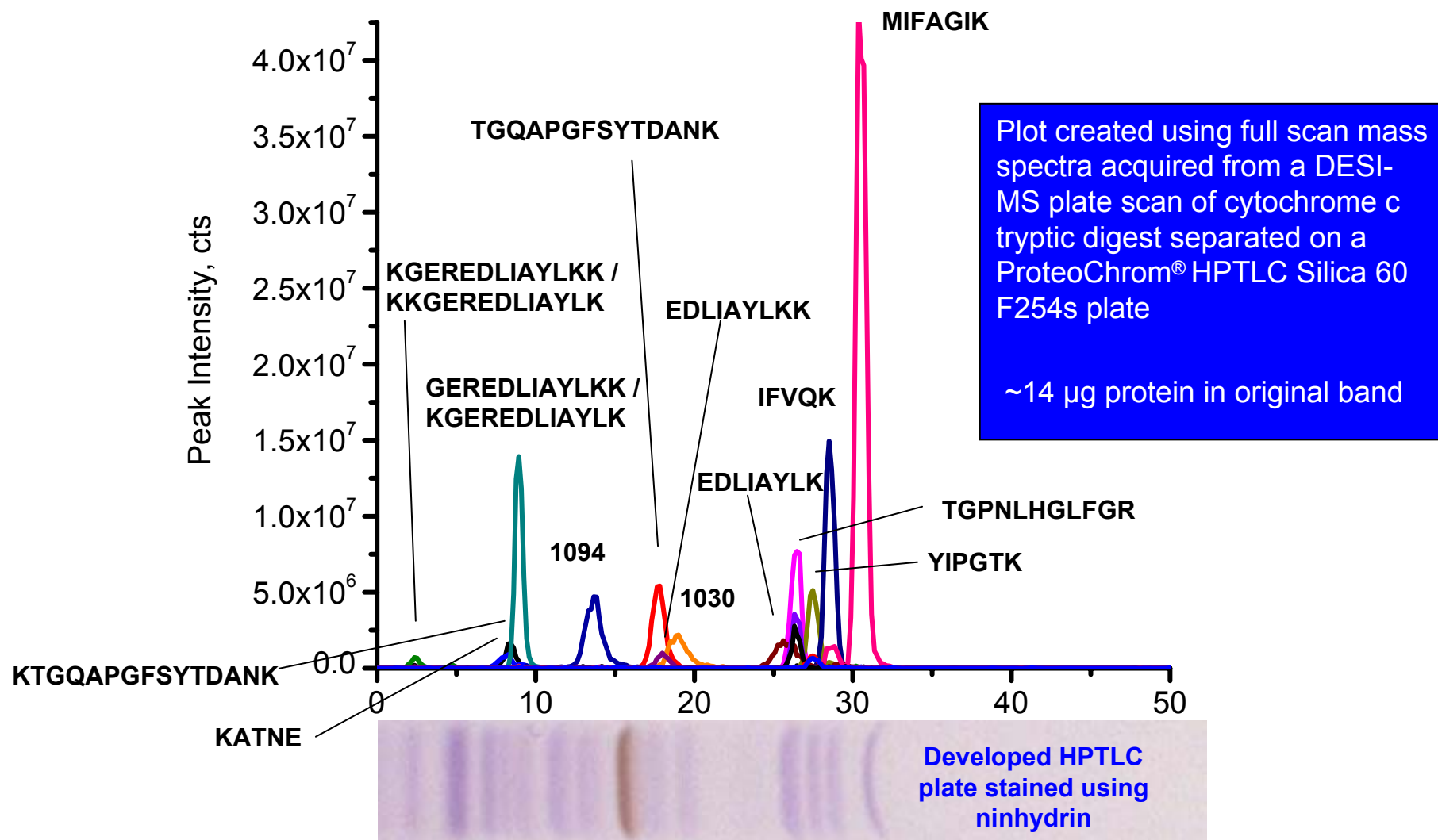
⁴ Label values also includes 10.7 mg hydrastine/capsule.

TLC/DESI-MS Spot Sampling



- 200 ng each rhodamine B, 6G and 123 in separate development lanes (RP C8 plate)
- full scan mass spectra acquired
- automated sampling of selected spots in each lane
 - 1 mm/s x, y, z movement
 - surface lowered 3 mm between spots
 - 20 s sampling at each spot
- 10 μ L/min methanol spray solvent
- 3.5 min analysis of 6 bands

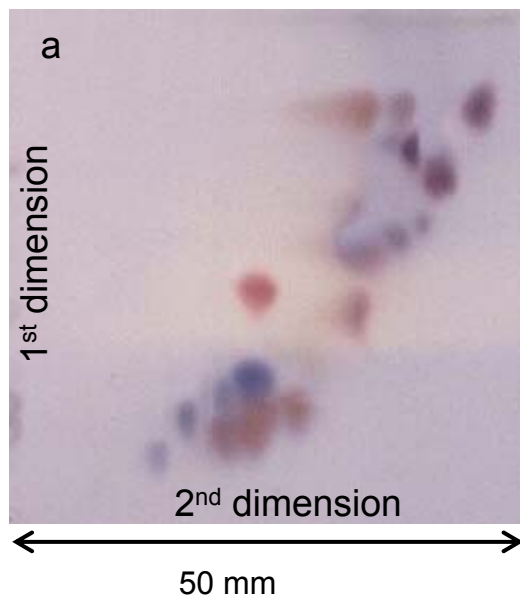
Using HPTLC/DESI-MS for Peptide Identification in 1D Separation of a Tryptic Protein Digest



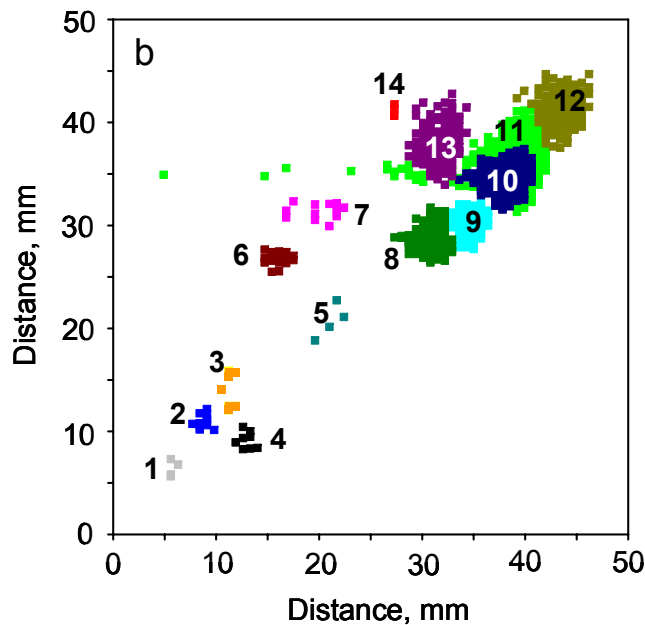
HPTLC/DESI-MS Imaging of a Tryptic Protein Digest Separated in 2D

Peptide distribution for a cytochrome c tryptic digest separated on a ProteoChrom® HPTLC Cellulose sheet.

Stained HPTLC sheet
(ProteoChrom® Color Peptide Stain)



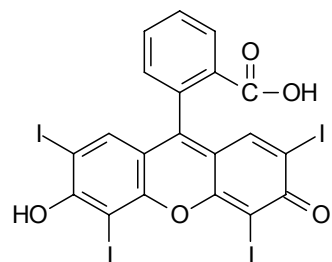
2D map created from MS/MS spectra acquired during sequential plate scans (imaging)



Peptide	ID
KKGER	1
KGER	2
KATNE	3
KGK	4
KTGQAPGFSYTDANK	5
KKGEREDLIAYLK	6
KGEREDLIAYLK	7
KYIPGTK	8
KIFVQK	9
TGPNLHGLFGR	10
IFVQK	11
MIFAGIK	12
EDLIAYLK	13
GITWGEETLMEYLENPK	14
Sequence coverage, from MS/MS data	80.0%

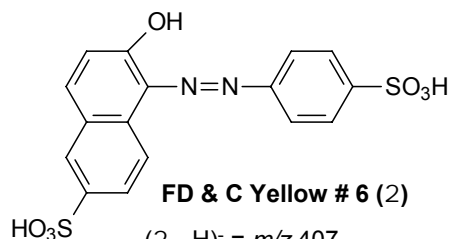
TLC/DESI-MS: Wetable RP C18

Food Dyes



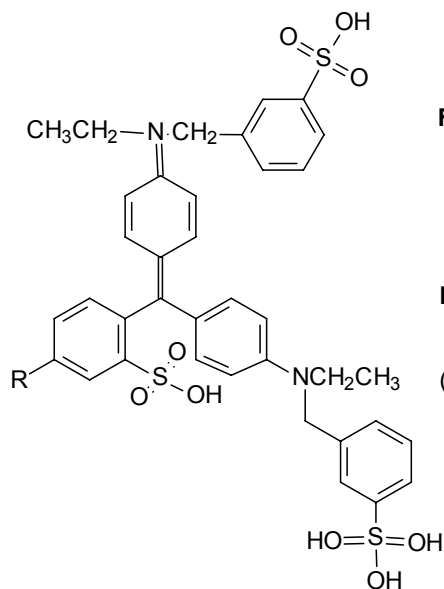
FD & C Red # 3 (1)
 $(1 - H)^- = m/z 834$

240 ng



FD & C Yellow # 6 (2) **320 ng**

$(2 - H)^- = m/z 407$
 $(2 - 2H + Na)^- = m/z 429$



FD & C Green # 3 (3) **250 ng**

R = OH

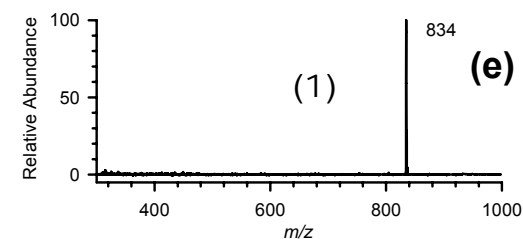
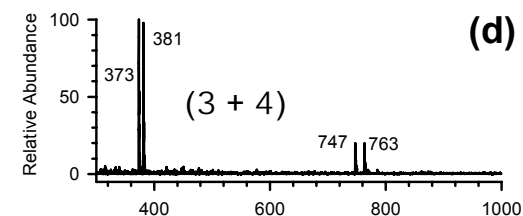
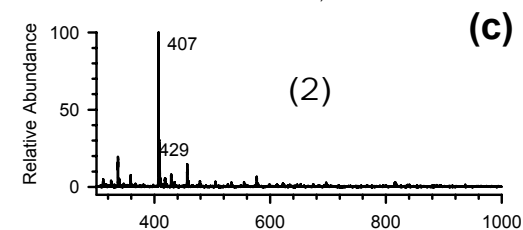
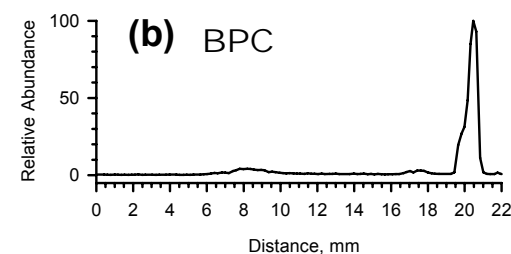
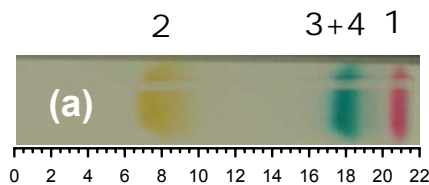
$(3 - 2H)^{2-} = m/z 381$
 $(3 - H)^- = m/z 763$

FD & C Blue # 1 (4) **260 ng**

R = H

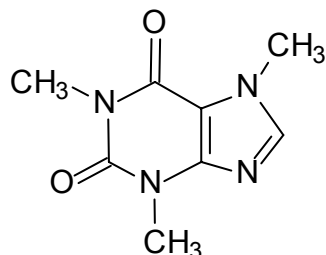
$(4 - 2H)^{2-} = m/z 373$

- full scan (EMS) negative ion mode data
- 190 $\mu\text{m/s}$ surface scan rate
- 10 $\mu\text{L/min}$ methanol spray solvent



TLC/DESI-MS: Normal Phase

Pharmaceuticals

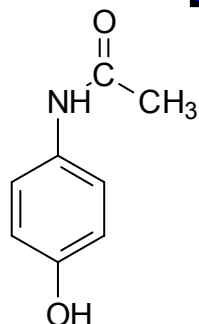


Caffeine (1)

$$(1 + H)^+ = m/z 195$$

$$(1 + Na)^+ = m/z 217$$

2 μ g

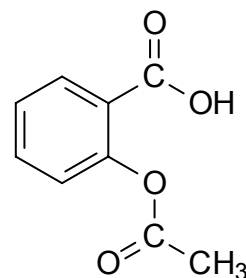


Acetaminophen (2)

$$(2 + H)^+ = m/z 152$$

$$(2 + Na)^+ = m/z 174$$

10 μ g

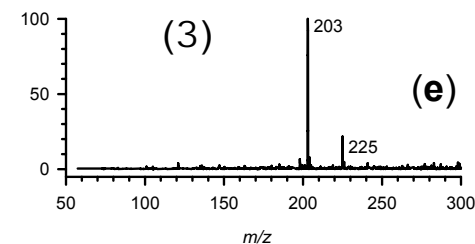
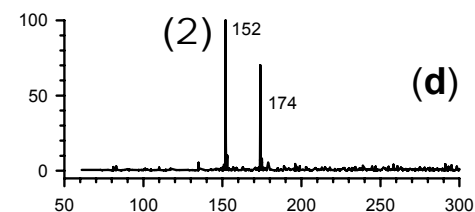
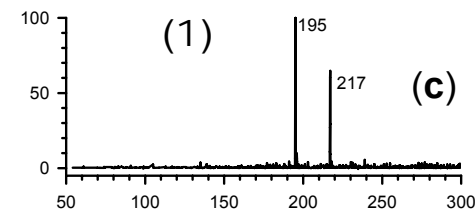
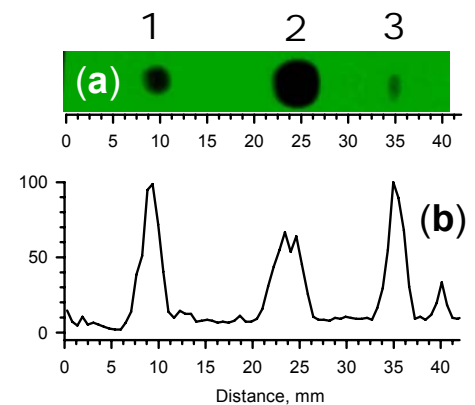


Aspirin (3)

$$(3 + Na)^+ = m/z 203$$

$$(3 + 2Na - H)^+ = m/z 225$$

10 μ g

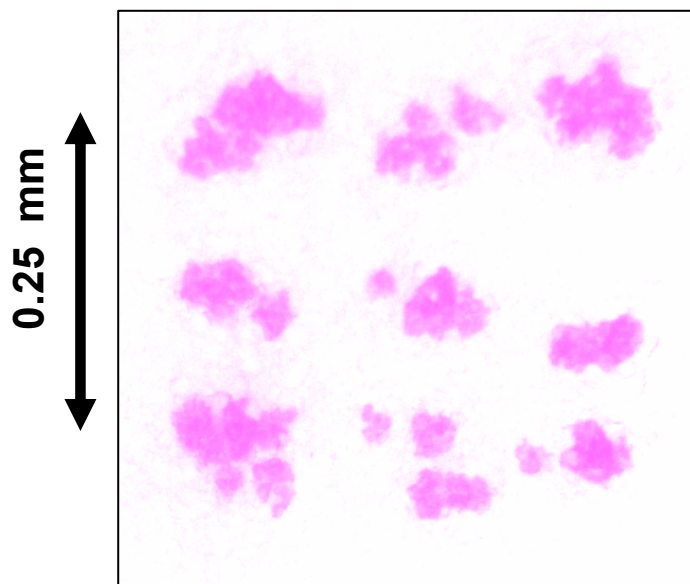


- aspirin, acetaminophen and caffeine in Excedrin tablets
- 190 μ m/s surface scan rate
- 10 μ L/min methanol spray solvent
- Desorption/ionization efficiency lower compared to reversed phase plates

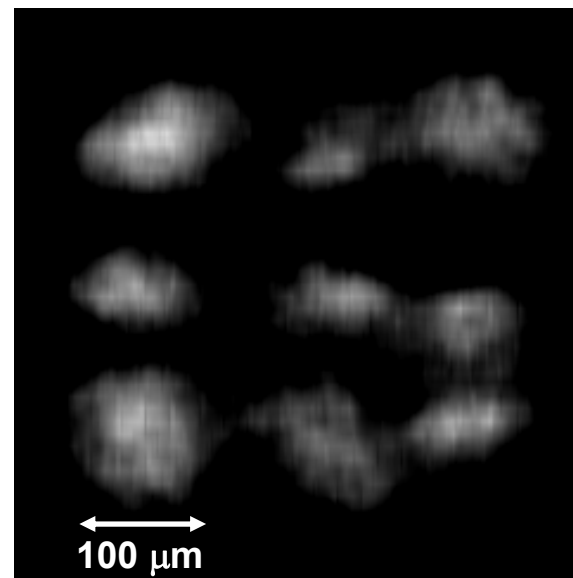
High-Resolution Imaging with DESI

0.25x0.25 mm Grid

Visible image

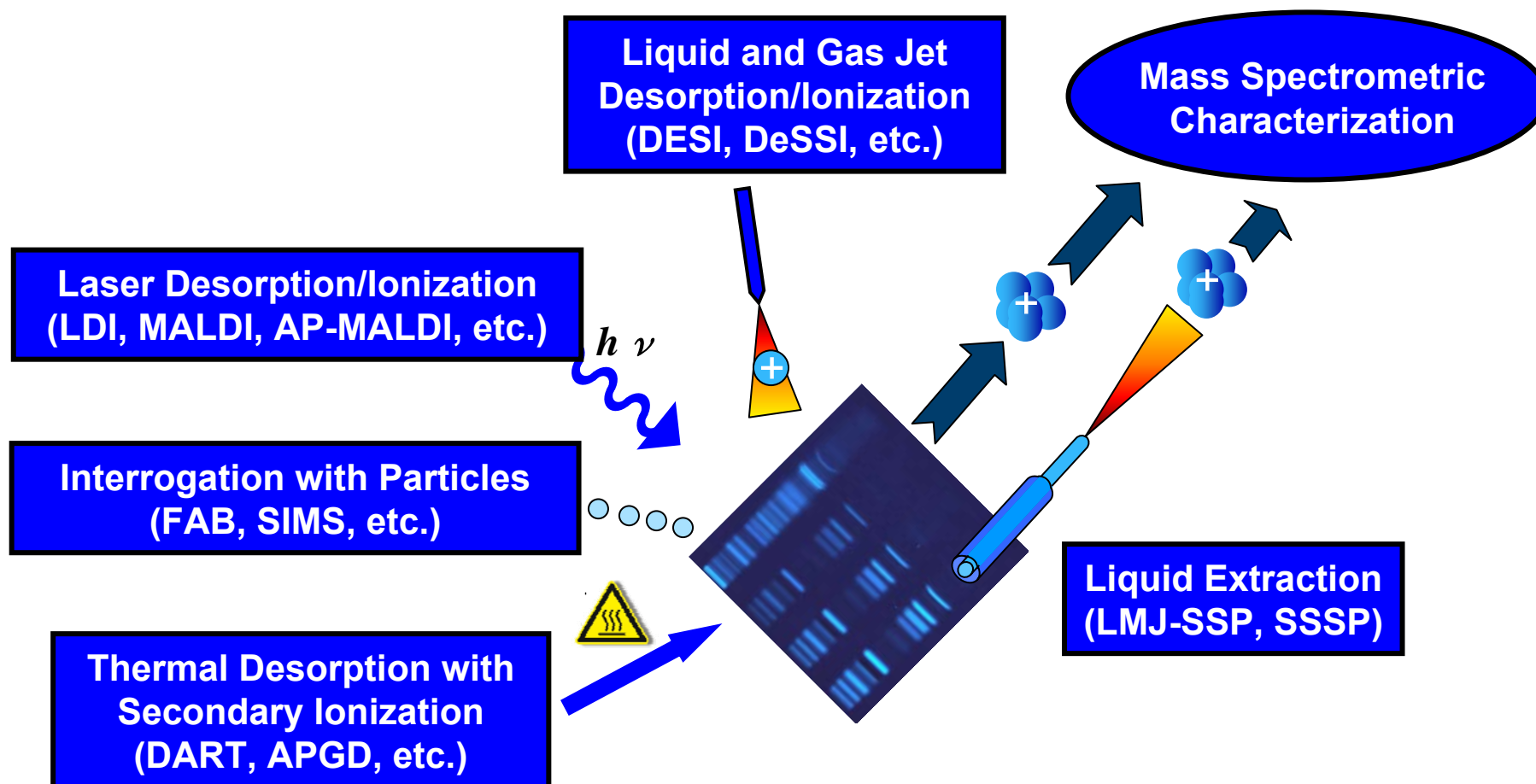


Chemical image



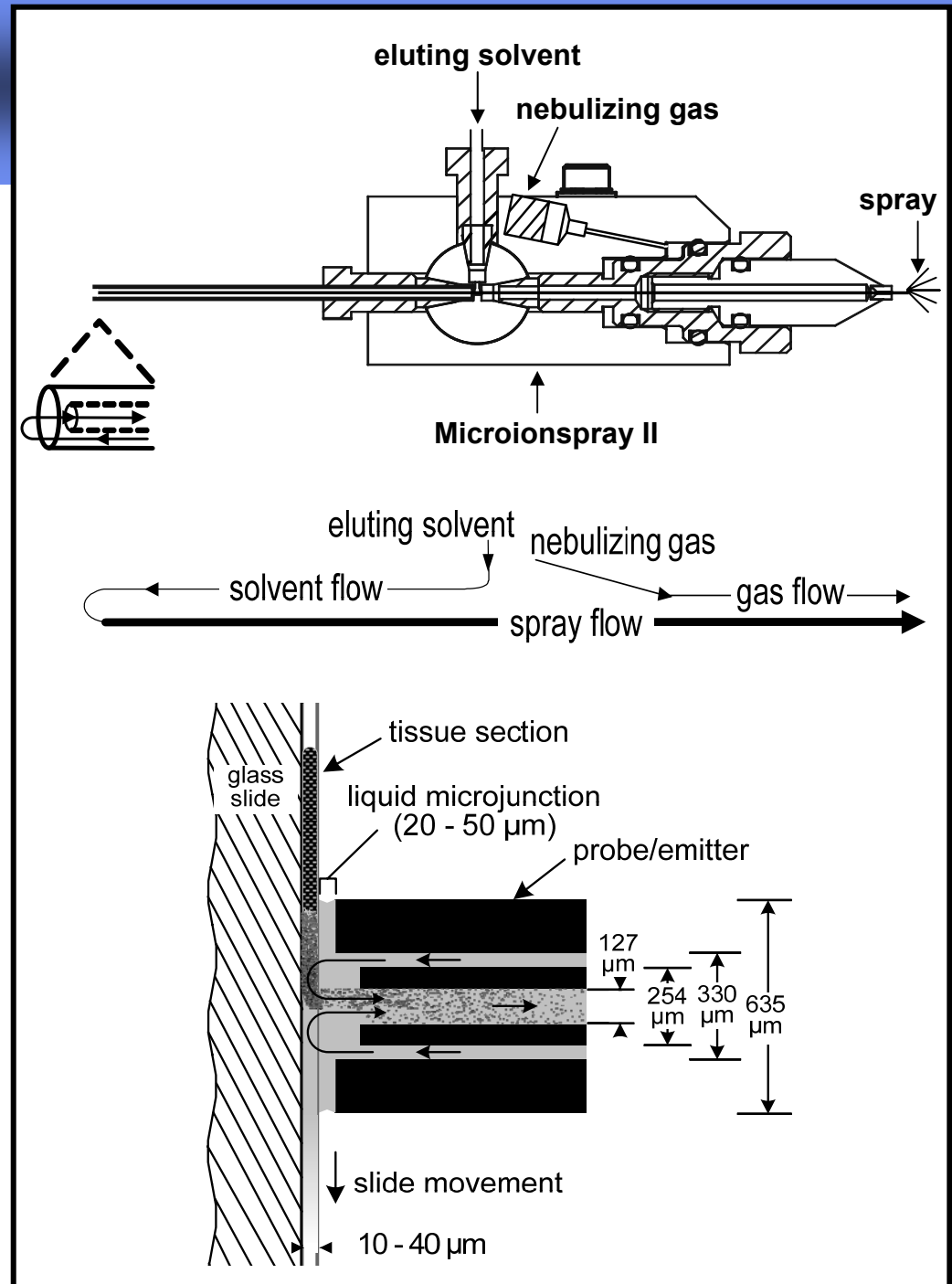
- 9 pixels positioned on the nodes of a 2x2 grid in 0.25x0.25 mm in size was printed on plastic back TLC plate using Epson magenta ink and was imaged using 1.5 μL/min acetonitrile as DESI spray solvent. Scan speed: 100 μm/s, step size: 10 μm, SRM: 601.5 → 303.5.
- Imaging resolution depends on plume size and desorption efficiency distribution within the DESI plume.
 - sub-plume-size resolution can be obtained
 - estimated resolution is ca. 40 μm

Atmospheric Pressure Surface Sampling/Ionization Methods for Mass Spectrometry



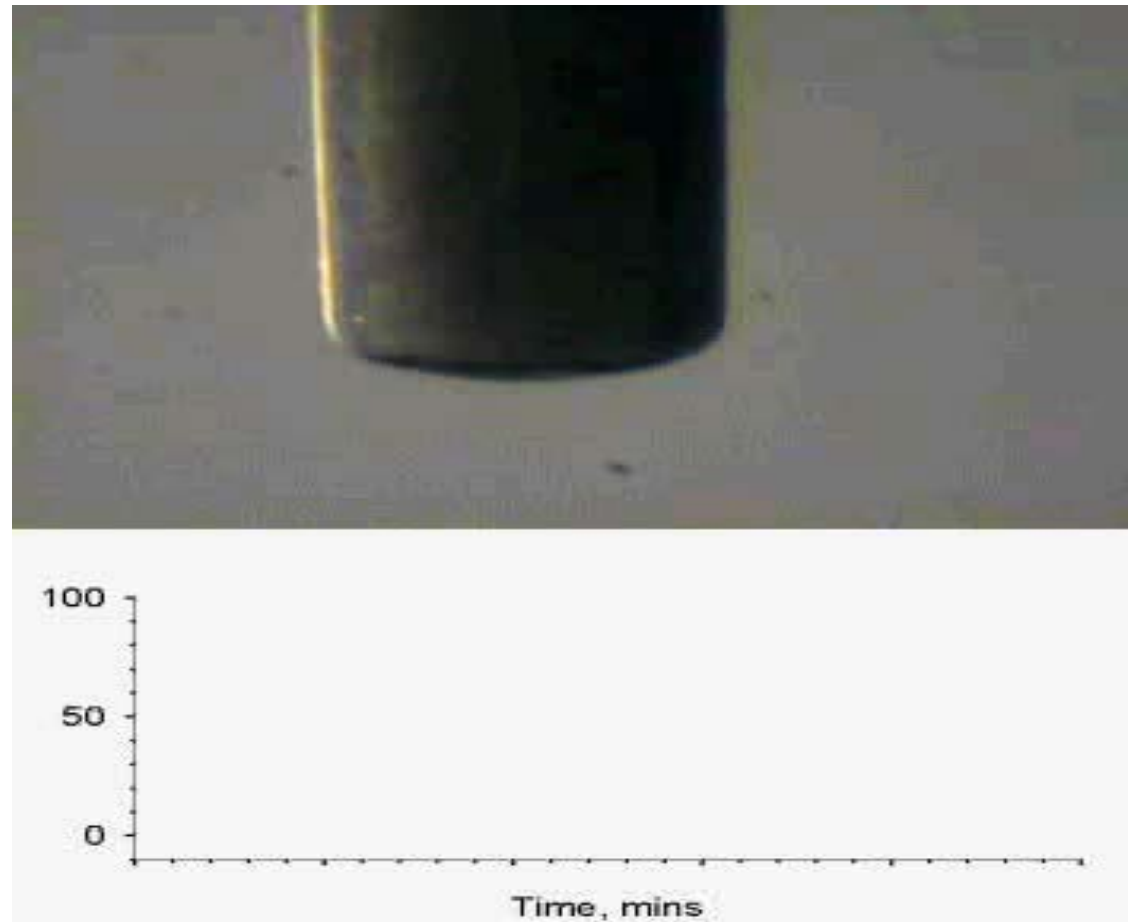
Liquid MicroJunction Surface Sampling Probe (LMJ-SSP)

- Eluting solvent pumped towards the surface through the annulus of the sampling and solvent delivery capillaries
- Solvent forms liquid micro-junction with surface
- Material from surface dissolved in solvent is aspirated from the surface through inner sampling capillary and sprayed
- Local pressure drop from pneumatic nebulizer used to aspirate solvent from the surface through inner sampling capillary



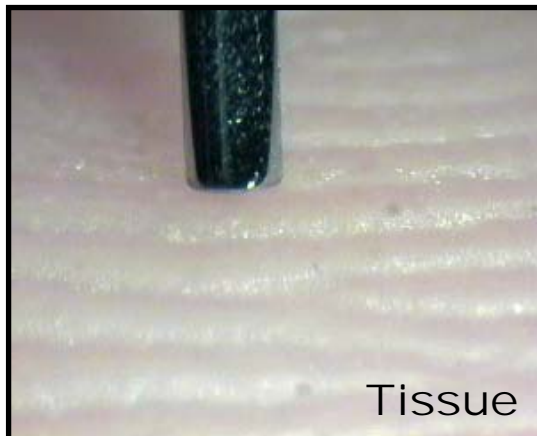
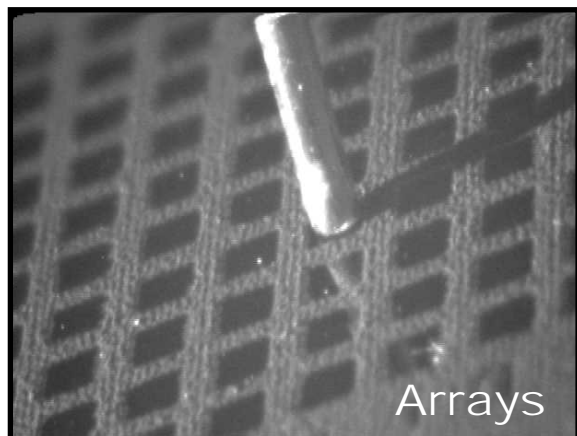
Surface Sampling Probe in Action

wash & vac



Liquid Micro-Junction Surface Sampling Probe (LMJ-SSP)

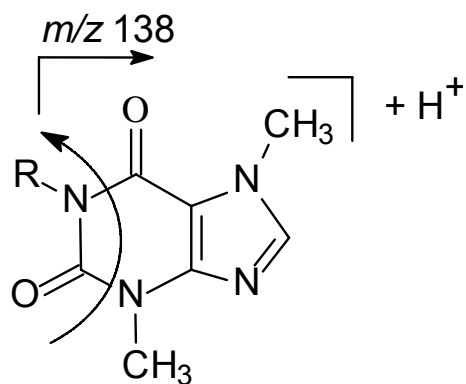
Self-aspirating probe for ambient analysis of analytes on a variety of surfaces coupled with ESI or APCI



Van Berkel, et al. *Anal. Chem.* 2002, 74, 6216-6223

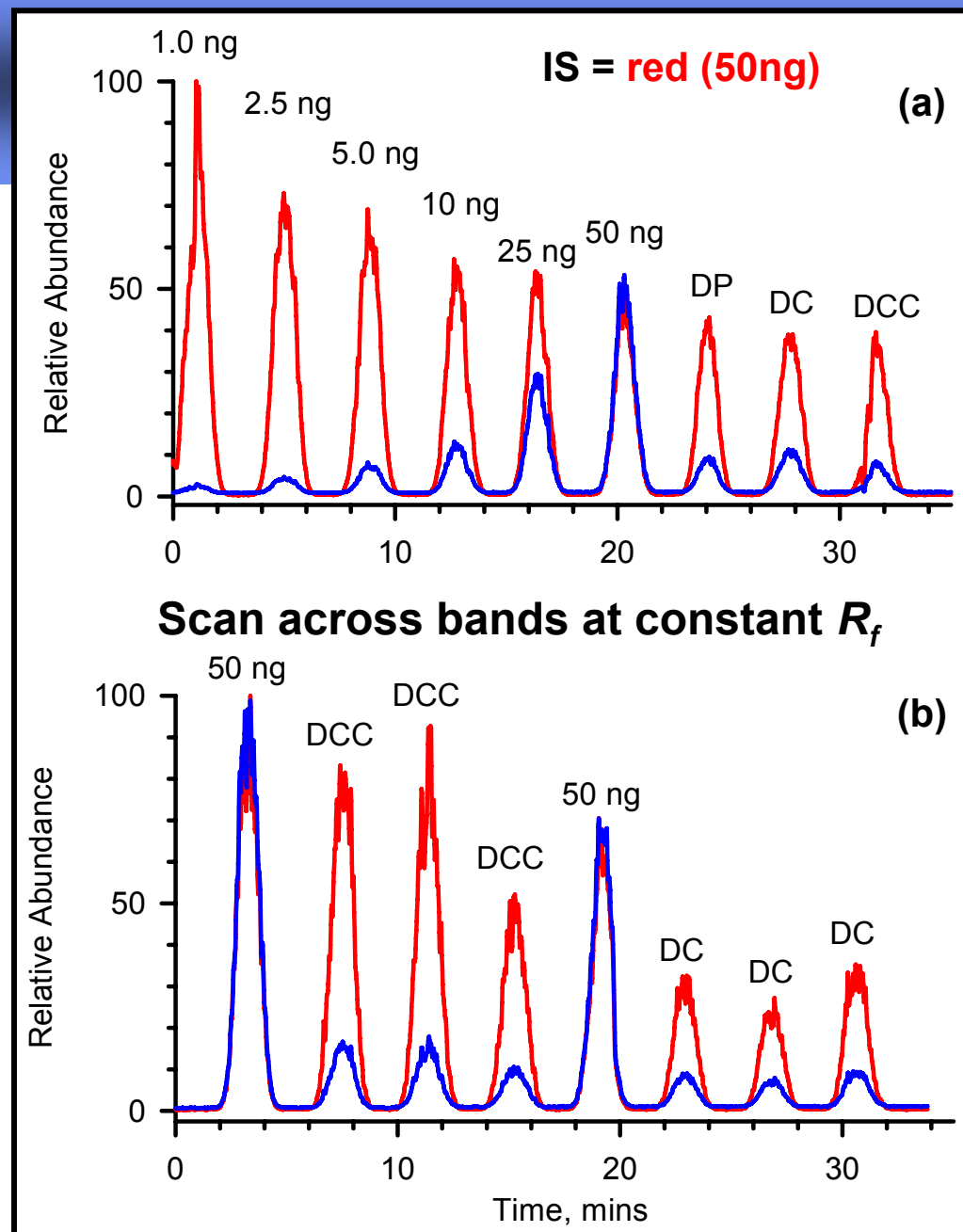
SSP: Quantitative TLC/ES-MS/MS

- Caffeine in diet beverages quantified using internal standard and selected reaction monitoring (SRM) detection



caffeine ($R = CH_3$)
 $(M + H)^+ = m/z$ 195

caffeine- d_3 ($R = CD_3$)
 $(M + H)^+ = m/z$ 198



SSP: Quantitative TLC/ES-MS/MS

Caffeine Determined in Six Beverages Using HPLC/UV and TLC/ES-MS/MS.

sample	lit ¹	caffeine per container, mg									
		HPLC/UV					TLC/MS				
		mean	mean vs lit (%)	std dev	%RSD	replicates	mean	mean vs lit (%)	std dev	%RSD	replicates
Diet Coke	45	46.0	+2.2	0.3	0.7	4	43.2	-4.0	0.7	1.6	3
Diet Pepsi	36	35.0	-2.8	0.2	0.7	4	34.7	-3.6	0.8	2.3	3
Diet Cherry Coke	34	36.9	+8.5	0.3	0.9	4	38.0	+12	0.8	2.1	3
Diet Turbo Tea®	90	120.8	+34	0.7	0.6	4	119.8	+33	0.8	0.7	3
Speed Stack™	250	276.2	+10	0.7	0.2	4	270.0	+8.0	11	1.5	3
Grape Speed Stack™	250	284.4	+14	0.7	0.25	4	278.0	+11	4.3	4.4	3
Fruit Punch											

¹ Literature values for Diet Coke, Diet Pepsi, and Diet Cherry Coke taken from ref. 15. Quoted values for other beverages taken from the manufacturer's label.

Summary

- Automated spot sampling, lane scanning, imaging of analytes separated on TLC plates
- Software interface for selection of sample spots, lane scan and raster scan parameters (e.g., surface scan speed, lane separation, distance, etc.)



DESI

- Can be used with all TLC plate separation phases
- Detection levels are sufficient for typical plate loadings (better than low ng DL range)
- Quantitative analysis (golden seal)
- Some care must be taken to avoid damaging separation phase (solvent limits on some phases)
- Applicable to species that work by ESI



LMJ-SSP

- Can be used with RP TLC plate separation phases
- Detection levels are sufficient for confident identification of low ng loadings
- Quantitative analysis (caffeine in soft drinks)
- Harder to automate and to handle the formation of the LMJ
- True ESI

DESI – Current State, Imaging

Handfree TLC/MS v2.30 - 2004.10.18 - ©2004 UT-Battelle, LLC working in DESI mode

Tools Large picture

Settings: Surface/water scan | Spot sampling | Area sampling

Parameters for lines to scan:
X-axis = 12 mm
Y-axis = 5 mm
Scan no. = 25
Lane step = 0.25 mm
Calculate scan no.

Eliminate heterogeneous spray effects
 Scan only in one direction
Speed X/Y = 1 mm/s
Speed Z = 1 mm/s
Lift off sprayer by 2 mm
End-start Z difference 0 mm


Lines to cont. Optimal dist.: 34 pixels Gap size: 50

Monitored color: Red Green Blue


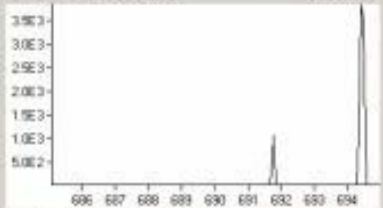
Filespec: C:\HD\Handfree\LDMS\5510_34_2004_A110_1_58.txt New

Actual position
X = 5.0000 mm Y = 2.5000 mm Z = 0.0000 mm Zero Calibration

Surface/MS image: Display Both Surface Image Refresh surface X=4.27 mm, Y=4.36 mm



Real time mass spectrum: collected Sun spectrum: Showing Selected area Actual spot



Sampling width = 0.1 mm, height = 0.25 mm

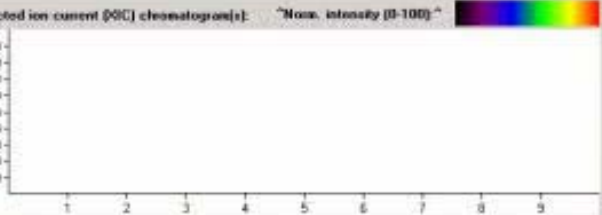
Min. m/z	Max. m/z	Min. int.	Max. int.	Color
693.6	698.0	0	100	Red

MS file: C:\DESI\1023\Winon\1004a01.P01w
 Monitor MS (start acquire MS first) Save...

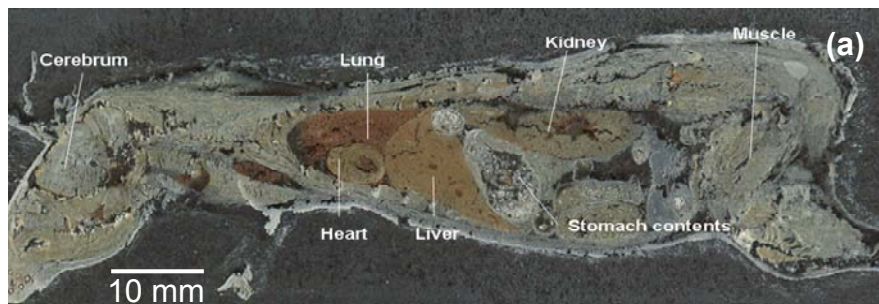
MS mode: Full scan SIM/MSM
Delay = 0

Buttons: Add, Edit, Remove, Refresh parameters/image, Load, Save, Sound, Move 50 um, Stop

Extracted ion current (EIC) chromatogram(s): Norm. intensity (0-100)

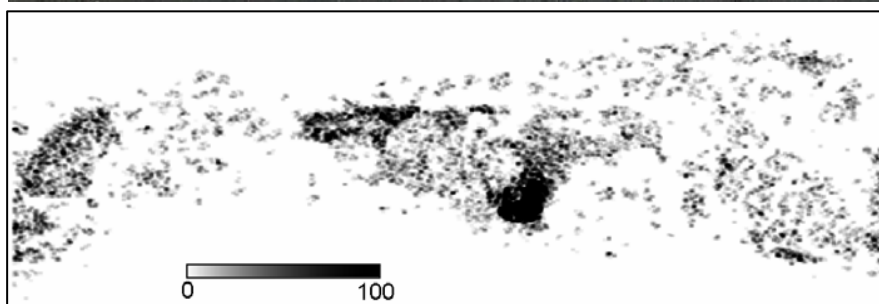


Chemical Image Comparison

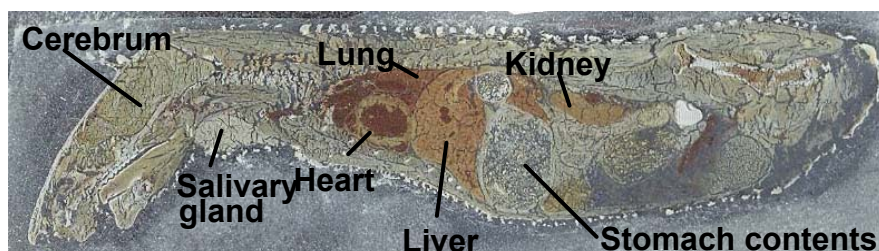


photo

60 min post 'cold'
Propranolol IV
Dose 7.5 mg/kg

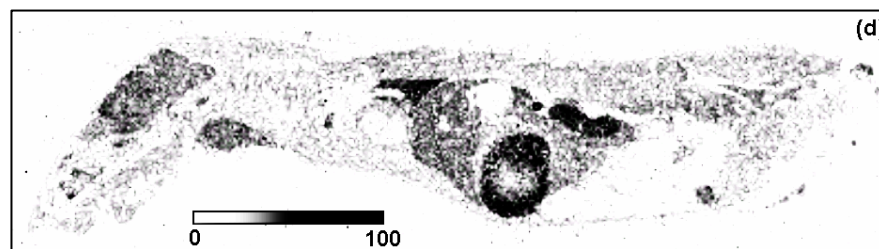


DESI-MS/MS Image
of Parent Drug



photo

60 min post
[³H]Propranolol IV
Dose 7.5 mg/kg



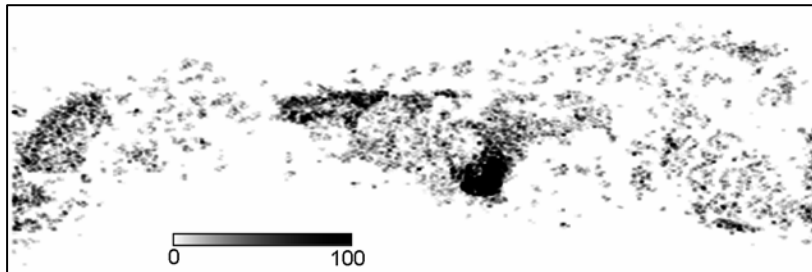
QWBA Image
Of Parent Drug
and Metabolites

- 40 µm sagittal sections
- SRM detection
- DESI solvent 80/20 (v/v) MeOH/H₂O at 5 µL/min
- 200 µm spaced lanes (151 lanes scanned)
- 7 mm/s scan rate, dwell time 20 ms

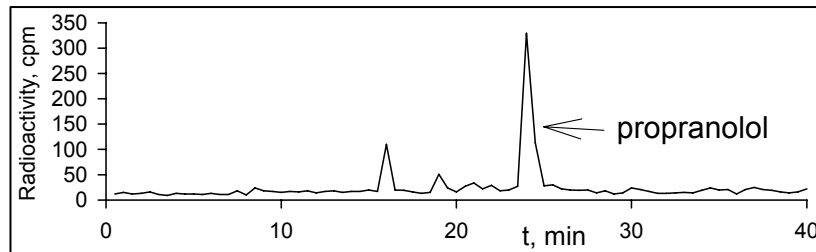
Quantitation

Comparison of radiolabel and DESI techniques (20 and 60 min post Propranolol IV Dose)

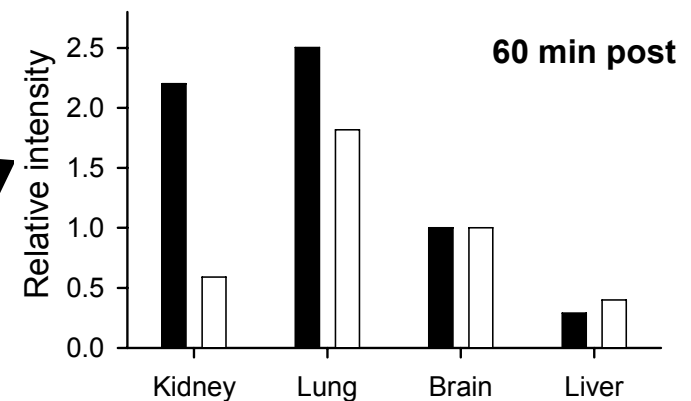
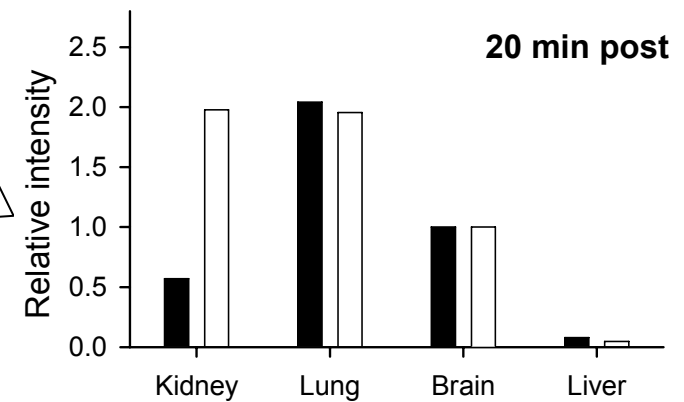
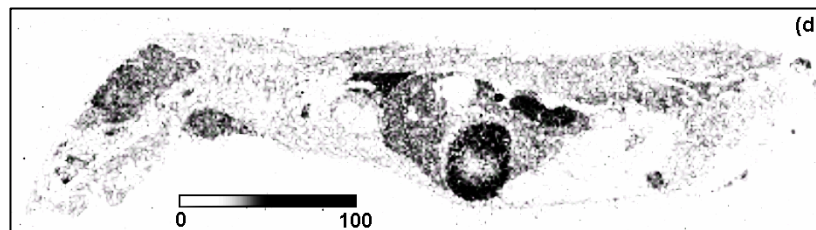
DESI: parent drug only



Radioprofilig: drug and metabolites distribution

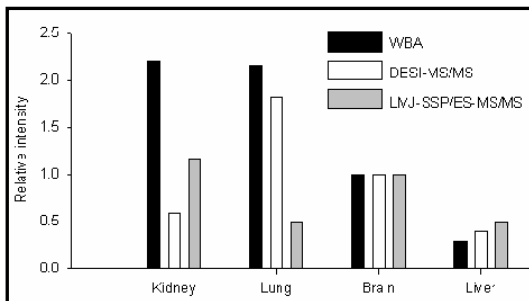


QWBA: parent drug and metabolites

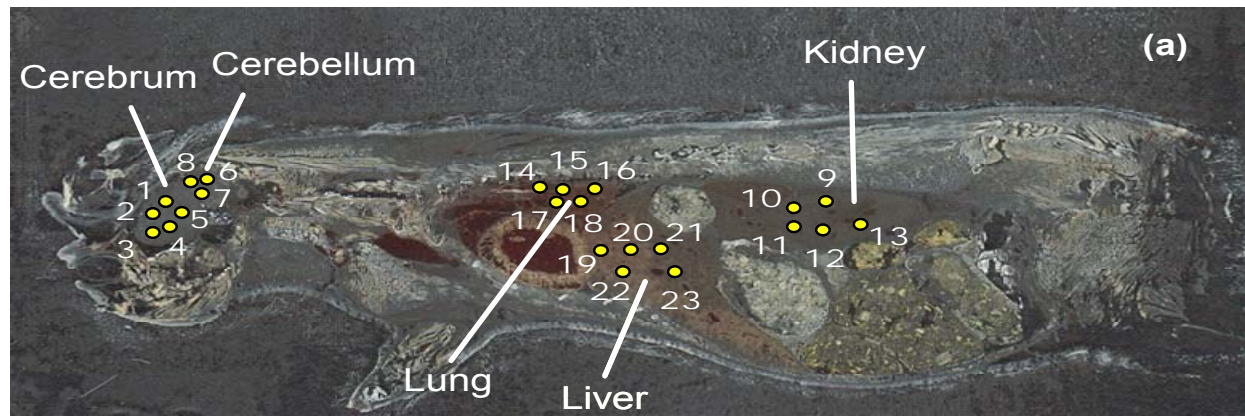


LMJ-SSP/ES-MS/MS Spot Sampling of Propranolol and its Major Metabolite

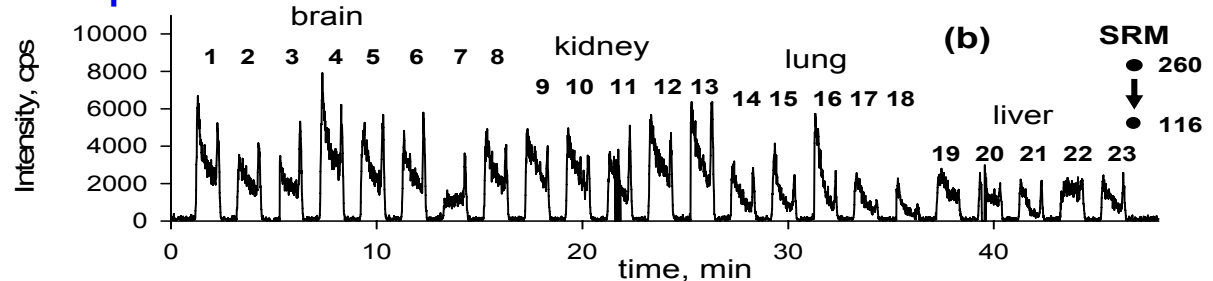
Propranolol levels: comparison of WBA, DESI-MS/MS and LMJ-SSP/ES-MS/MS



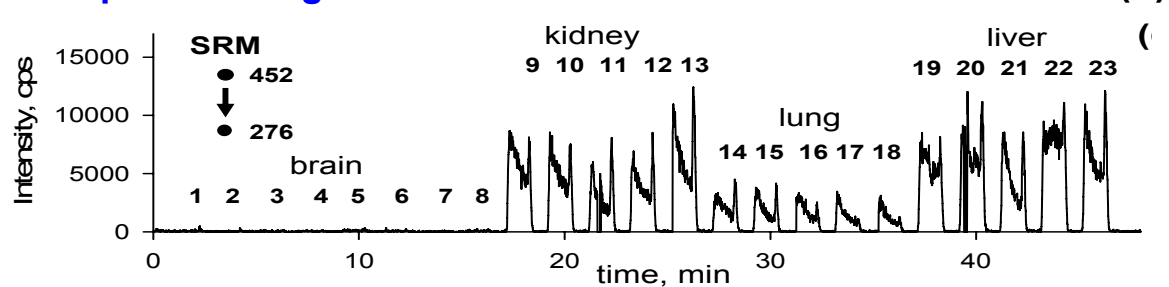
Known metabolite observed with LMJ-SSP/ES-MS/MS but not DESI-MS/MS



Propranolol



Propranolol-O-glucuronide



e.g., Van Berkel, et al. *J. Mass Spectrom.* 2008, 43, 500-508