

# Desorption Electrospray Ionization (DESI) Mass Spectrometry: Small Organics, Peptides and Proteins on Insulators and in Biological Tissues

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Ref. Takats, Wiseman, Gologan, Cooks  
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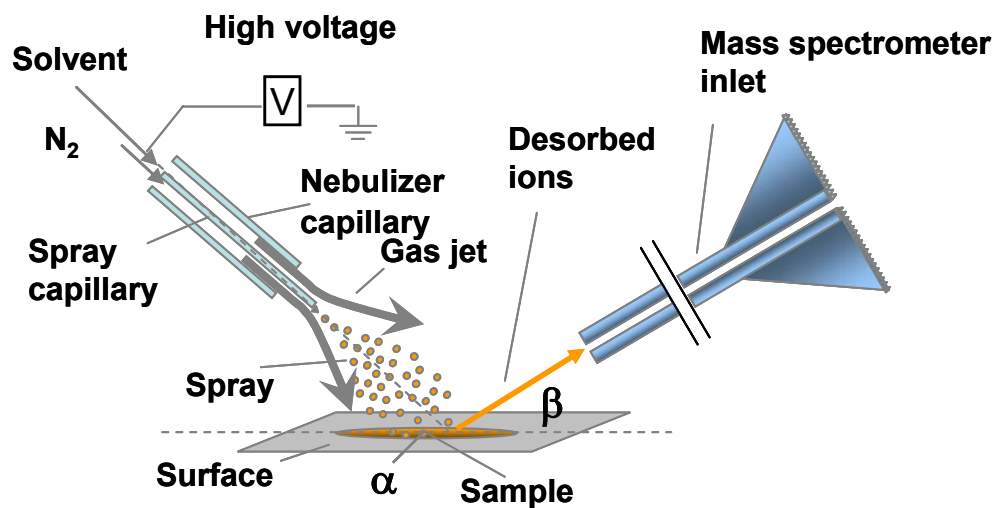
# Overview

- Instrumentation and analytical characteristics
- Chiral quantification
- Detecting explosives from complex mixtures
- High throughput tablet analysis
- Urine analysis without separation
- Desorbing peptides and proteins from insulators
- Profiling mammalian tissue

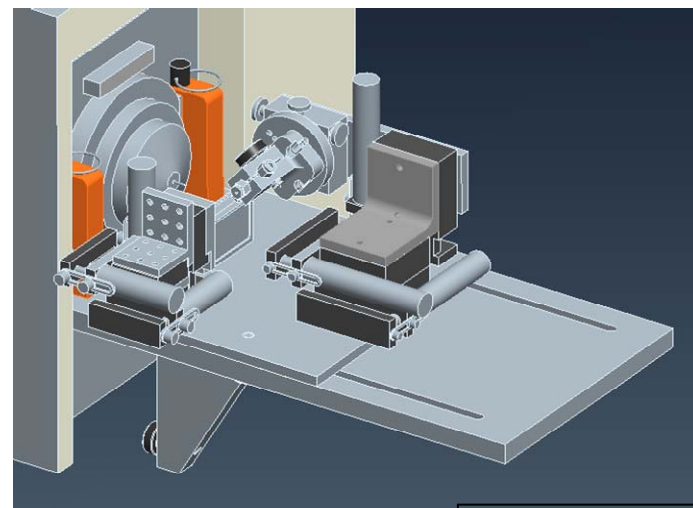
# Characteristics of desorption electrospray $M^*$

- Small organics through biological polymers
- Ambient conditions: full access to sample during MS
- Absolutely no sample preparation
- Very short analysis times (< 5 sec incl. MS/MS)
- *In situ* analysis of biological tissues
- *In vivo* analysis

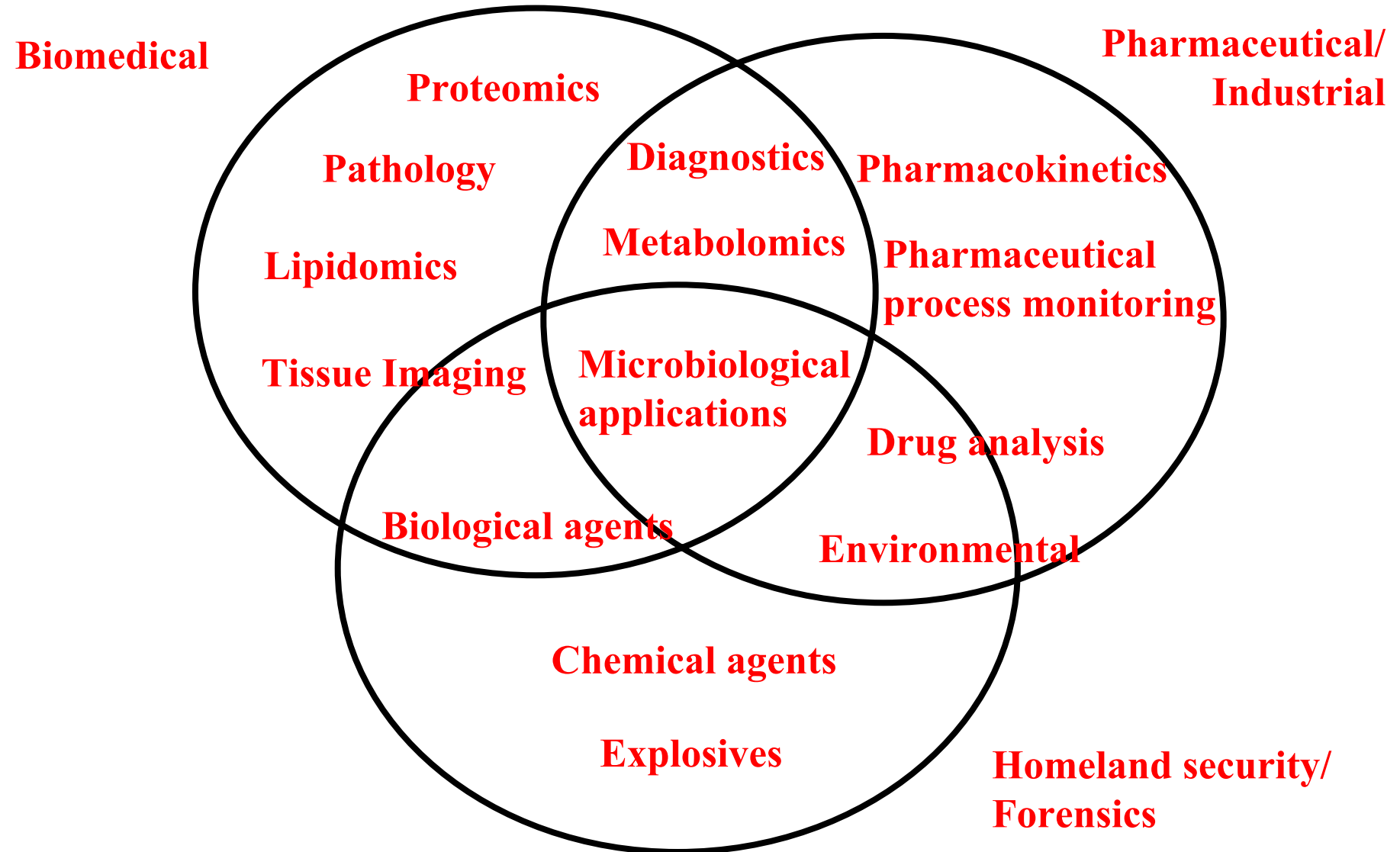
# Desorption electrospray ion source



| Parameter                    | Value      |
|------------------------------|------------|
| Voltage                      | 4-5kV      |
| N <sub>2</sub>               | 80-120 psi |
| incident angle ( $\alpha$ )  | 45-80°     |
| collection angle ( $\beta$ ) | 5-10°      |
| tip-to-sample distance       | 1-5 mm     |
| tip-to-inlet distance        | 5-8 mm     |



# Applications



# Chiral quantification by DESI-MS

- **Advantages**

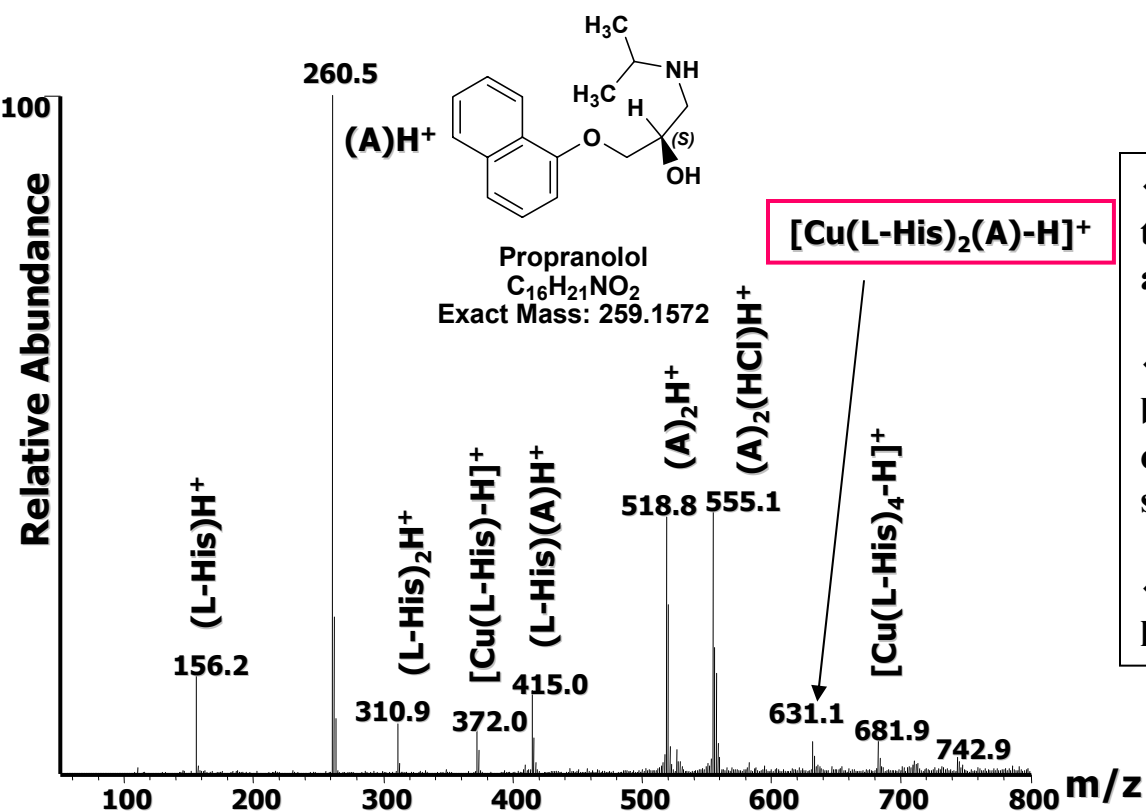
- Limited sample prep
- Uses small amounts of sample
- No need for a chromophore
- No need for a chiral column
- Faster method for ee analysis

- **Limitations**

- Not as precise as traditional chiral methods
- Possible isobaric interferences
- Reference availability
- Cluster formation
- $R_{\text{chiral}} > 1.2$
- Desorption efficiency

# Chiral analysis using the kinetic methods by DESI: $M^*$ R-Propranolol Quantification

Surface = Cotton Swab with S-Prop (A)  
Solvent =  $\text{CuCl}_2$ , L-His, Water pH 10



Kinetic method formalism

$$\ln \frac{k_1}{k_2} = \ln \frac{[\text{M}(\text{A}_{\text{R,S}})(\text{ref}^*)]^+}{[\text{M}(\text{ref}^*)_2]^+} = \frac{\Delta(\Delta\text{G})}{RT_{\text{eff}}}$$

❖ Diastereomeric clusters are generated through the use of analyte enantiomers, chiral reference and a metal ion.

❖  $\Delta(\Delta\text{G})$  is the difference in free energies between the two reactions in the non-reference channel and is directly related to the degree of steric hindrance in a chiral cluster

❖ The energy difference is proportional to the log of the product ion abundance ratio

DESI mass spectrum of  $\text{CuCl}_2(\text{M})$  and L-Histamine (ref\*) with S-Propranolol (A) on a cotton swab dissolved in 50/50 ME<sub>2</sub>O/H<sub>2</sub>O. The spectrum was recorded on LCQ DECA XP. The trimeric cluster ion is at  $m/z$  631.

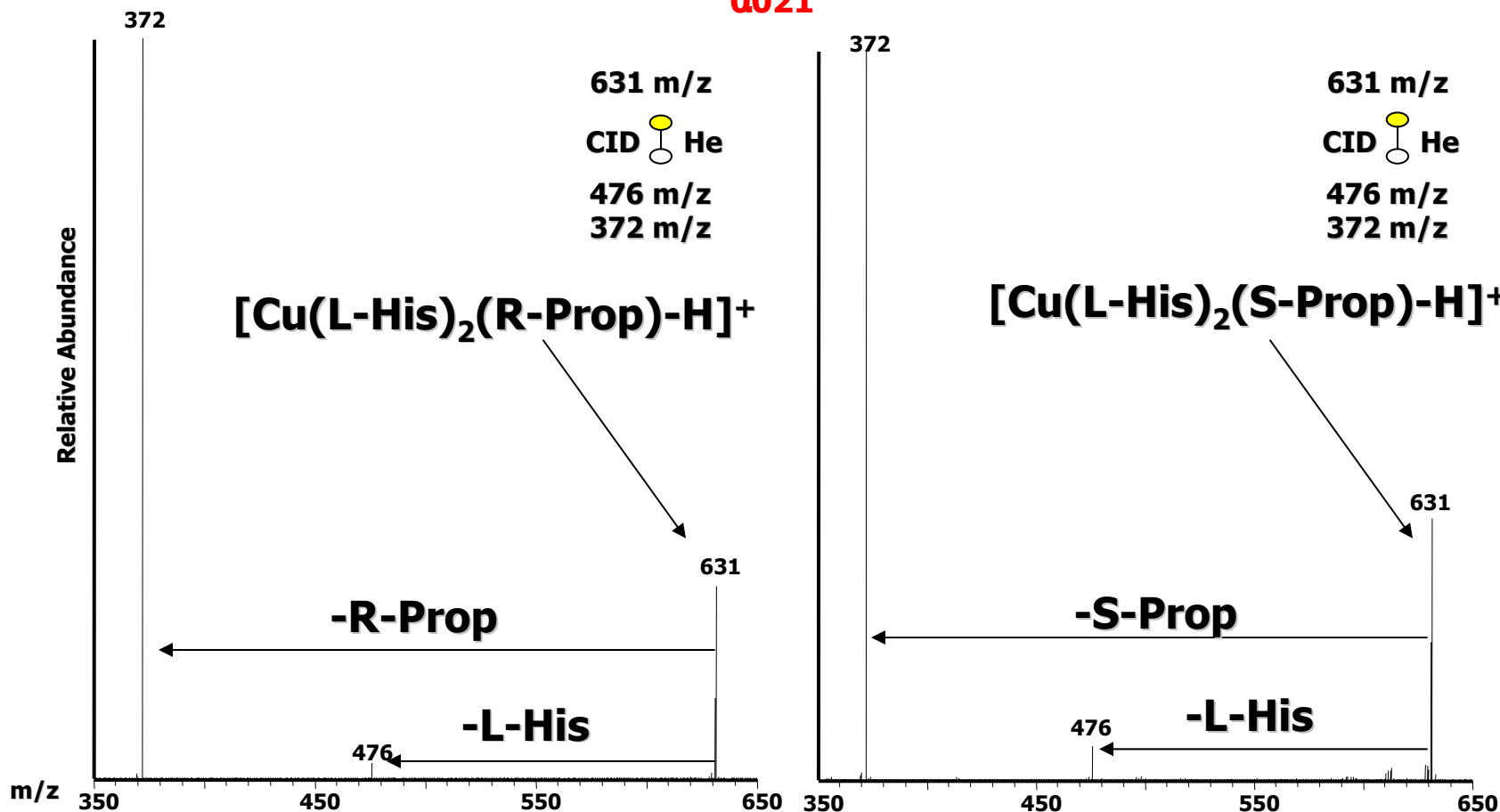
# Chiral analysis: Tandem Mass Spectrometry

$M^*$

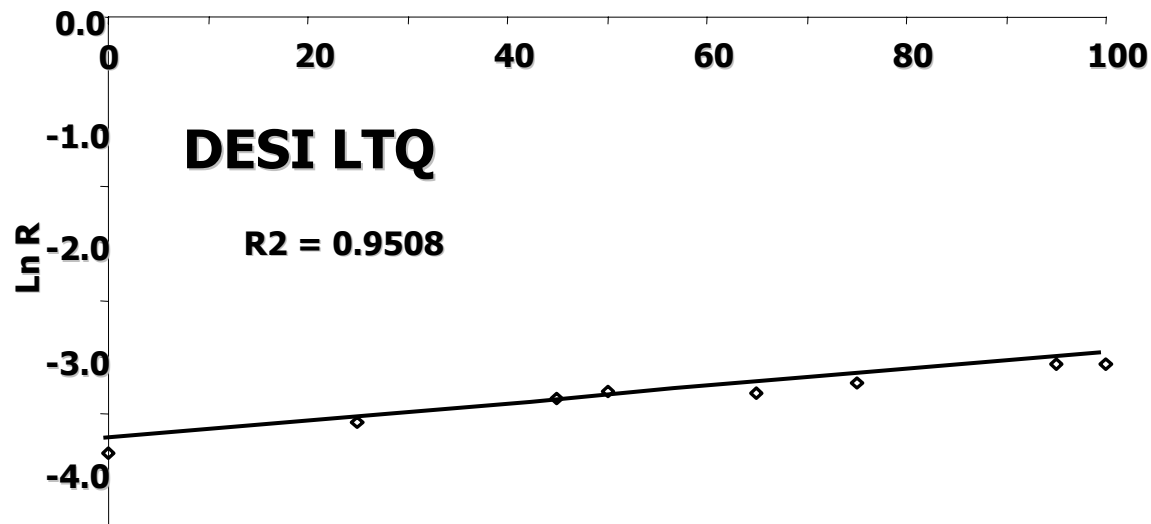
Chiral selectivity

$$R_{chiral} = \frac{I_R/I_{ref^*}}{I_S/I_{ref^*}}$$

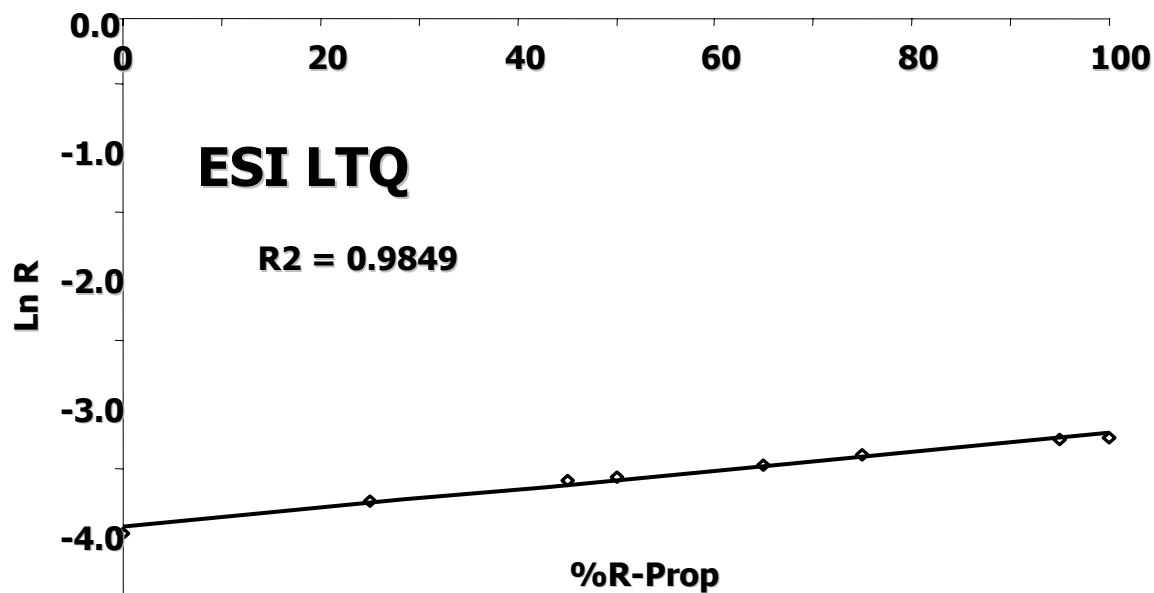
$$R_{chiral} = \frac{0.047}{0.021} = 2.20 \pm 0.14$$



# Calibration Plot for R-Propranolol $M^*$ Quantification



| Entry | Slope  | Y-intercept | R <sup>2</sup> |
|-------|--------|-------------|----------------|
| 1     | 0.0081 | 3.8249      | 0.9051         |
| 2     | 0.0071 | 3.7370      | 0.7474         |
| 3     | 0.0074 | 3.7521      | 0.8748         |
| Avg.  | 0.0075 | 3.7713      |                |
| SD    | 0.0005 | 0.0470      |                |
| %RSD  | 6.8119 | 1.2463      |                |



| Entry | Slope  | Y-intercept | R <sup>2</sup> |
|-------|--------|-------------|----------------|
| 1     | 0.0074 | 3.9585      | 0.9902         |
| 2     | 0.0072 | 3.9525      | 0.9684         |
| 3     | 0.0073 | 3.9498      | 0.9890         |
| Avg.  | 0.0073 | 3.9536      |                |
| SD    | 0.0001 | 0.0045      |                |
| %RSD  | 0.7873 | 0.1126      |                |

# Unknown Chiral Purity Determination

*M\**

| <u>Actual</u>  | <u>Experimental</u> | <u>Experimental</u> | <u>Relative STD Error</u>     |
|----------------|---------------------|---------------------|-------------------------------|
| %R-Propranolol | DESI                | ESI                 | DESI (ESI)                    |
| 25.00          | 31.61               | 28.78               | 0.235 (0.367)                 |
| 50.00          | 58.81               | 54.46               | 0.321 (0.219)                 |
| 75.00          | 70.32               | 76.79               | 0.761 (0.261)                 |
| 100.00         | 97.42               | 95.48               | 0.168 (0.217)                 |
| .....          |                     |                     |                               |
| 45.00          | -----               | 50.51               | ----- (0.528)                 |
| 65.00          | 69.62               | 66.91               | 0.944 (0.554)                 |
| 70.00          | 69.28               | -----               | 0.254 -----                   |
| 85.00          | 87.56               | -----               | 1.944 -----                   |
| 95.00          | 97.45               | 92.95               | 1.763 (0.372)                 |
|                |                     |                     | <b>Average: 0.799 (0.360)</b> |

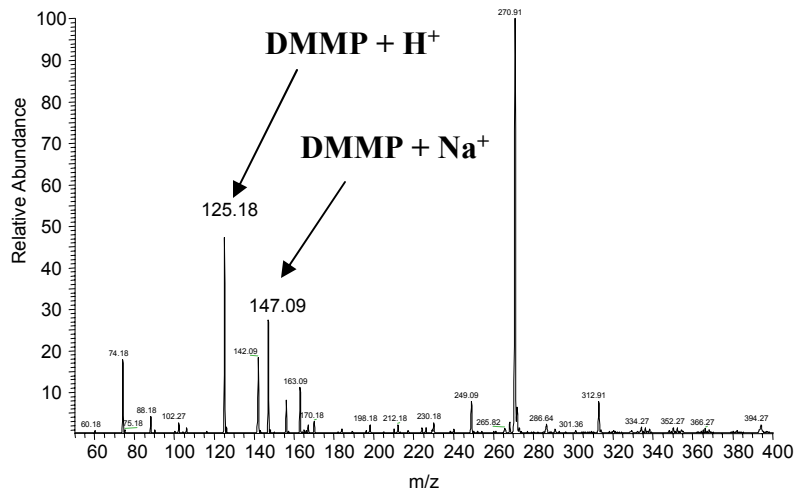
# Detecting explosives from surfaces in $\mathcal{M}^*$ complex matrices

- Provides real time surface analysis of a variety of surface types
- Sensitivity in picogram range for the tested chemicals
- Provides detection in complex matrices without sample treatment

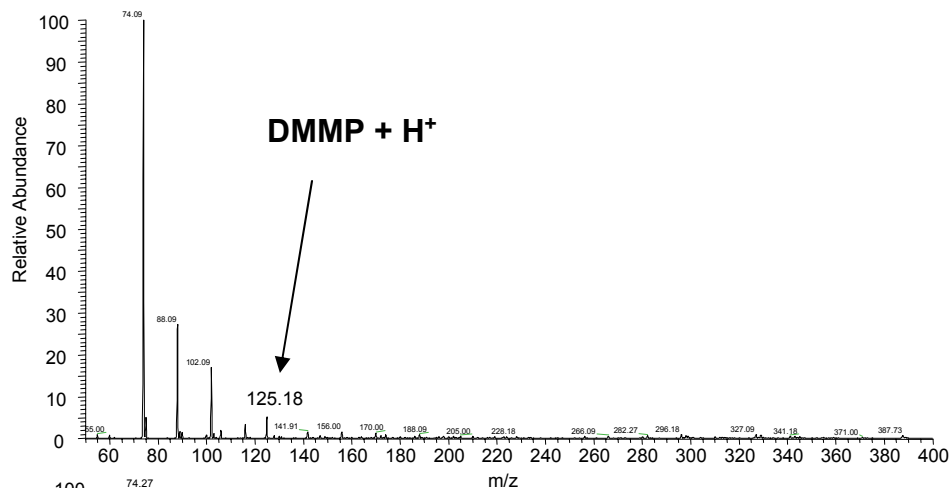
# Detecting explosive chemicals from complex matrices

$M^*$

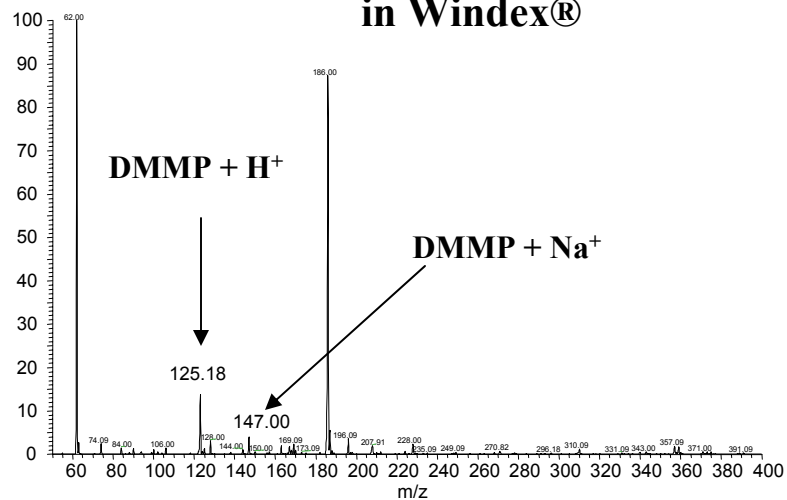
in diesel fuel



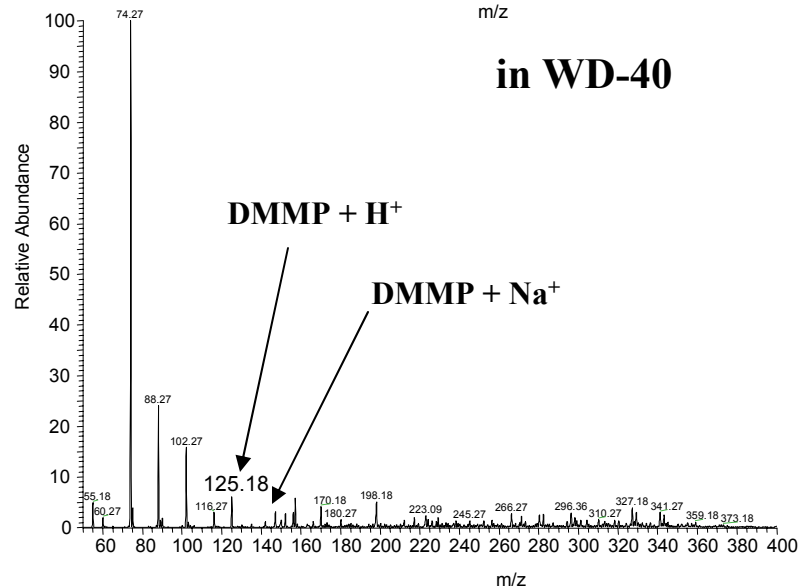
in Clorox® matrix



in Windex®



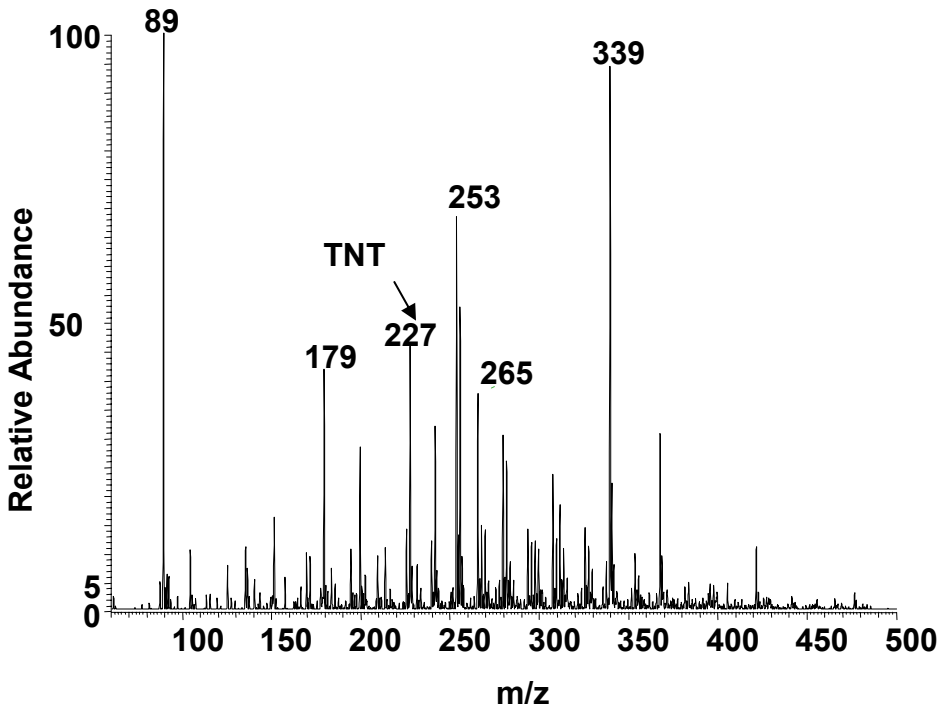
in WD-40



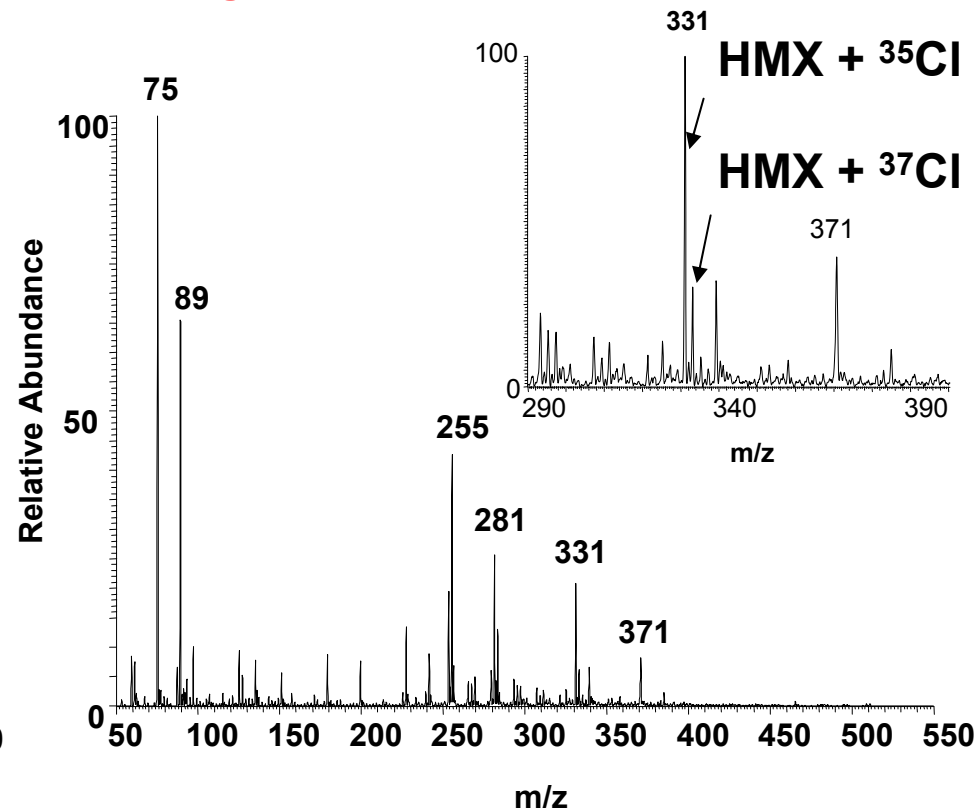
# Positive and negative ion detection of $\mathcal{M}^*$ explosives on natural surfaces

5 sec, in ambient environment

**Positive ion mode**  
10 fg TNT detected on paper using  
Methanol/water

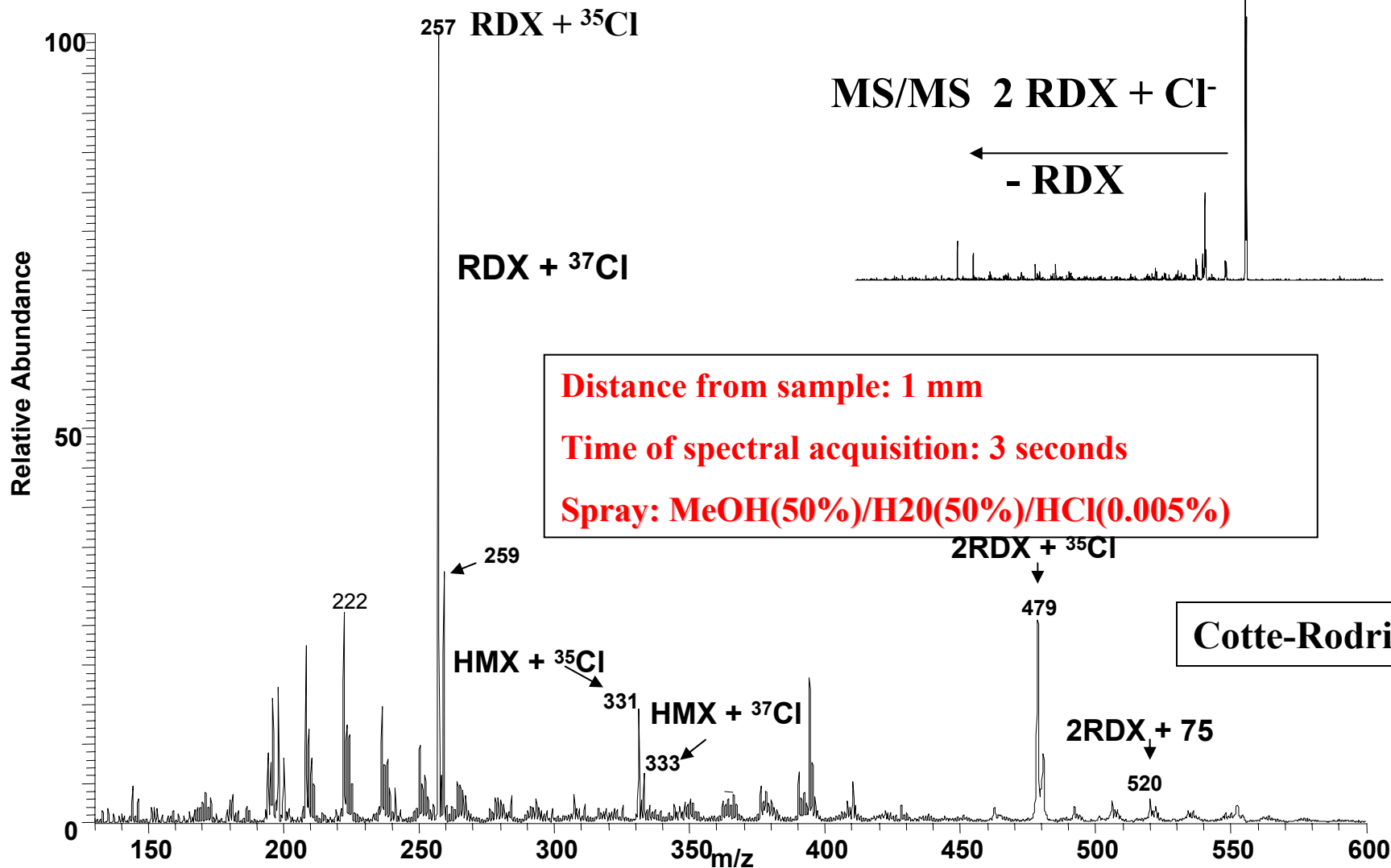


**Negative ion mode**  
100 pg HMX detected on cotton swab



# Plastic explosive C-4 on Teflon (10pg)

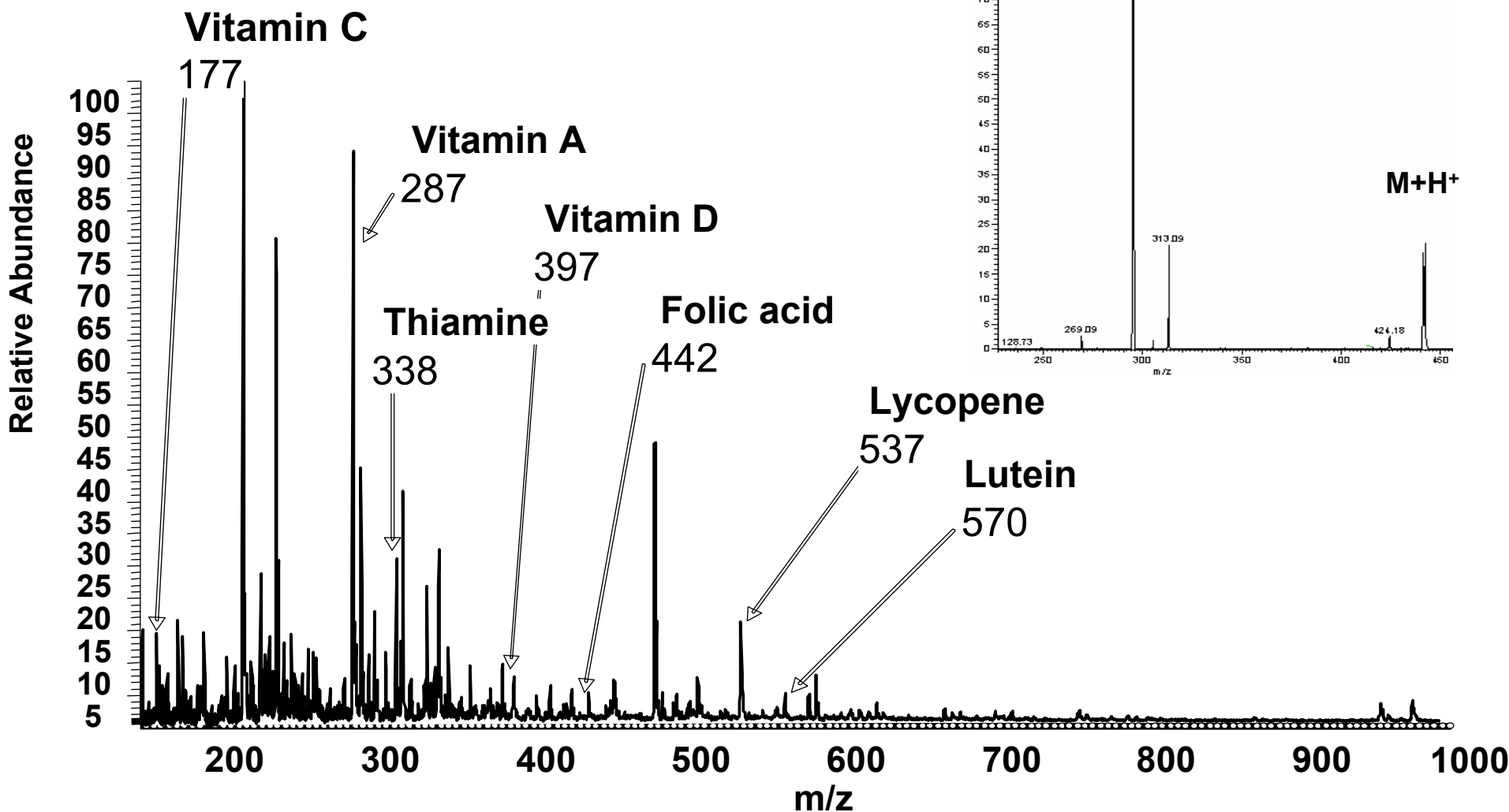
$\mathcal{M}^*$



# DESI-MS of Multi-vitamin tablet

$M^*$

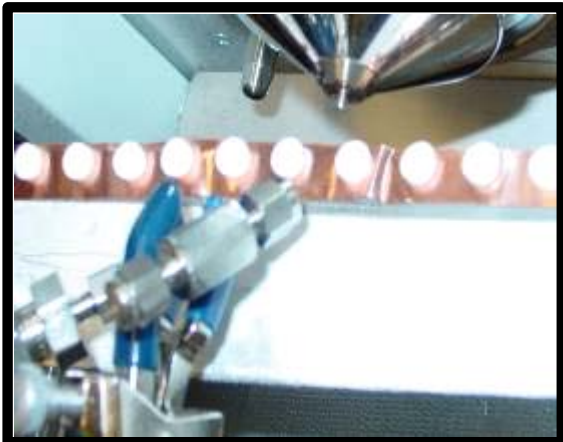
- Ambient sample
- Methanol/water spray; 5 sec analysis time



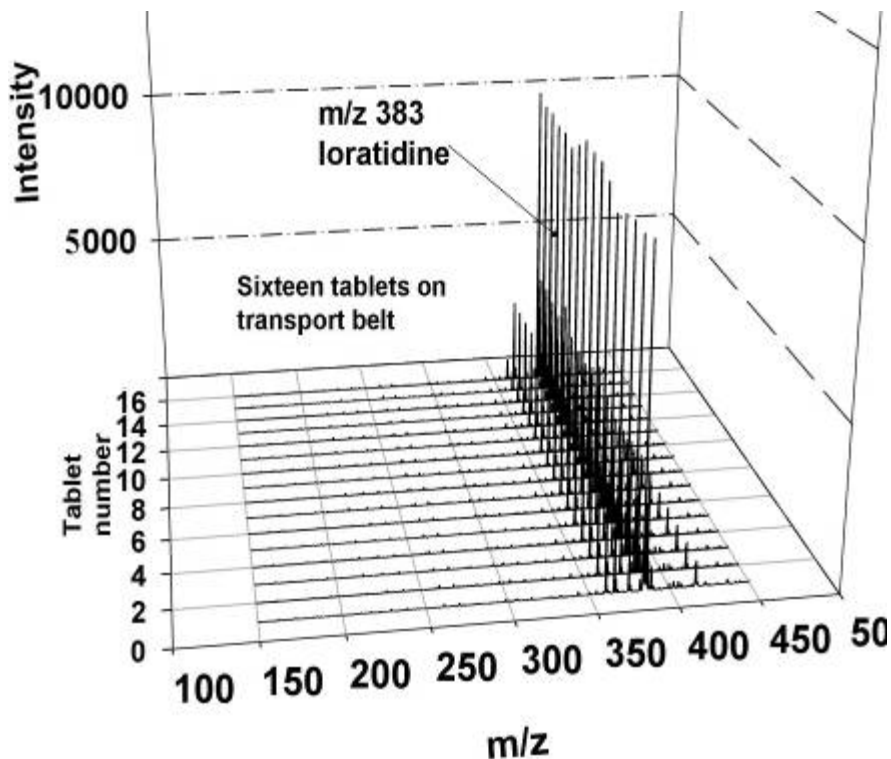
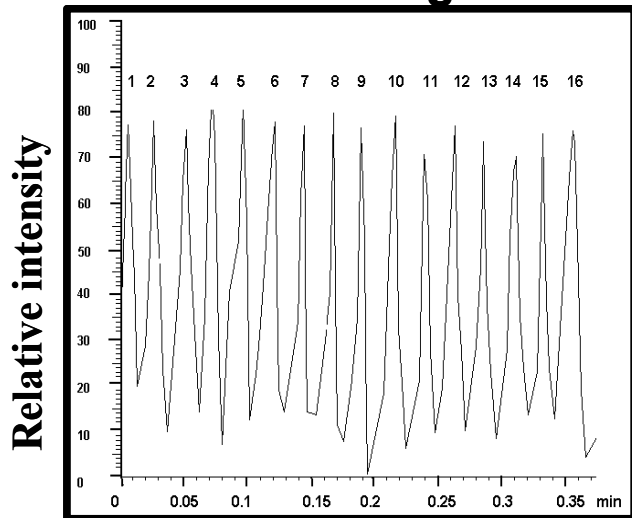
# High throughput tablet analysis

*M\**

## 16 Claritin® Tablets on transport belt



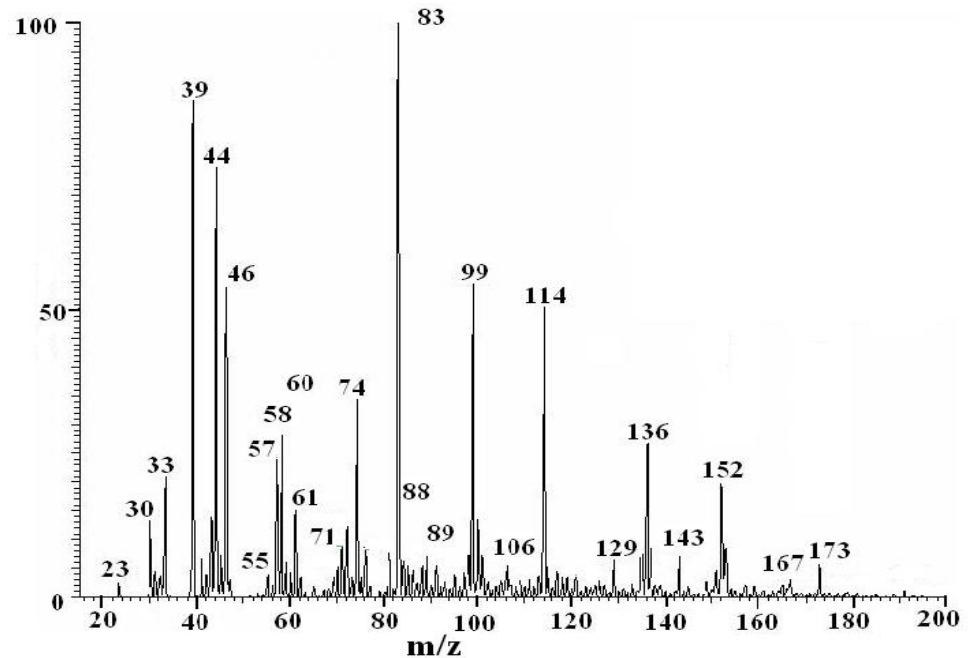
### Ion chromatogram



Sampling rate ~ .76 Hz  
% RSD < 3%

# Urine analysis without separation

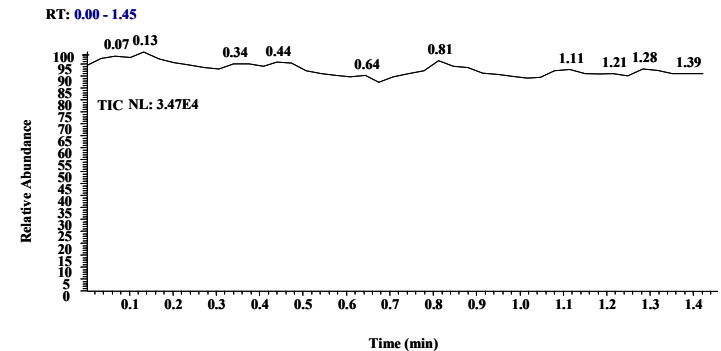
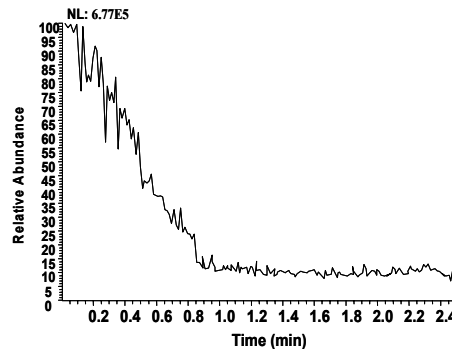
| <u>m/z</u> | <u>Peak assignment</u>     |
|------------|----------------------------|
| 61         | urea+H <sup>+</sup>        |
| 83         | urea+Na <sup>+</sup>       |
| 99         | urea+K <sup>+</sup>        |
| 106        | serine+H <sup>+</sup>      |
| 114        | creatinine+H <sup>+</sup>  |
| 136        | creatinine+Na <sup>+</sup> |
| 152        | creatinine K <sup>+</sup>  |



APCI with infusion

DESI of urine deposited on paper

**Salt tolerance**

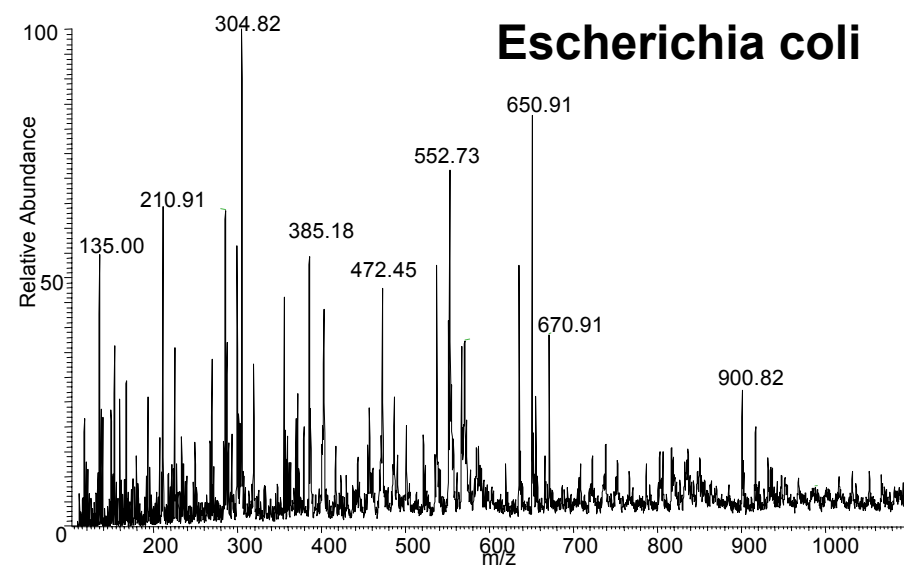
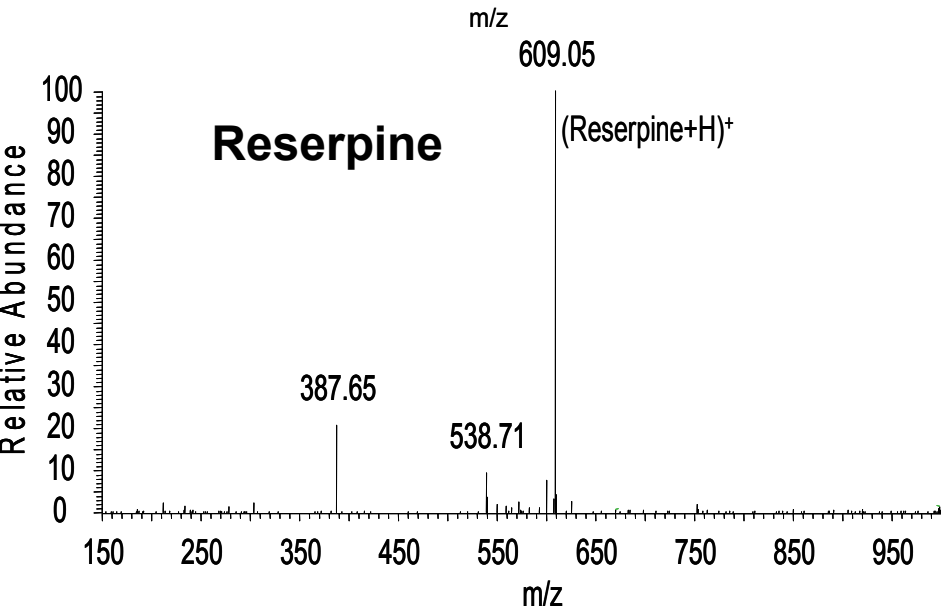
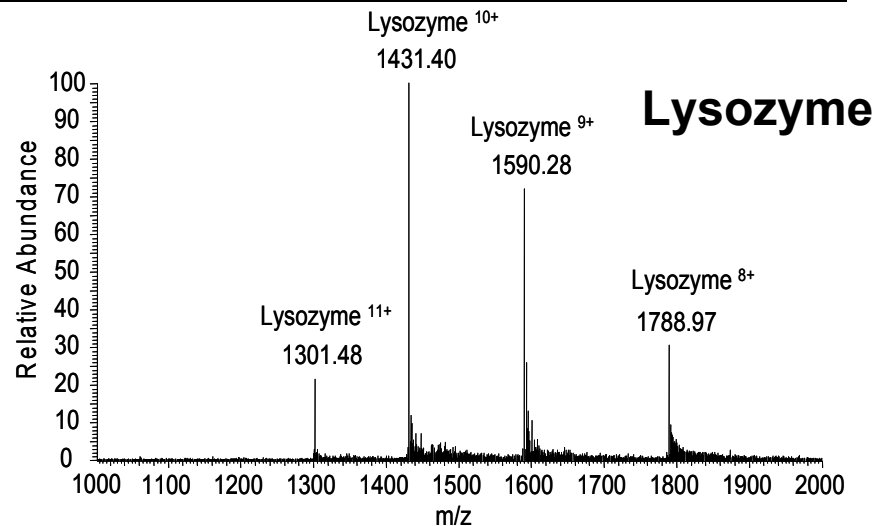
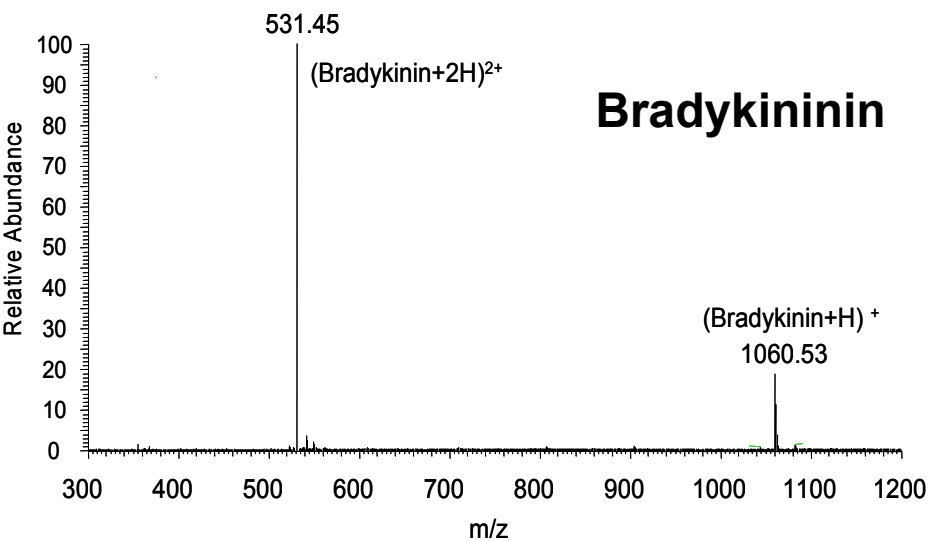


Signal drop down to 10% in 1 min

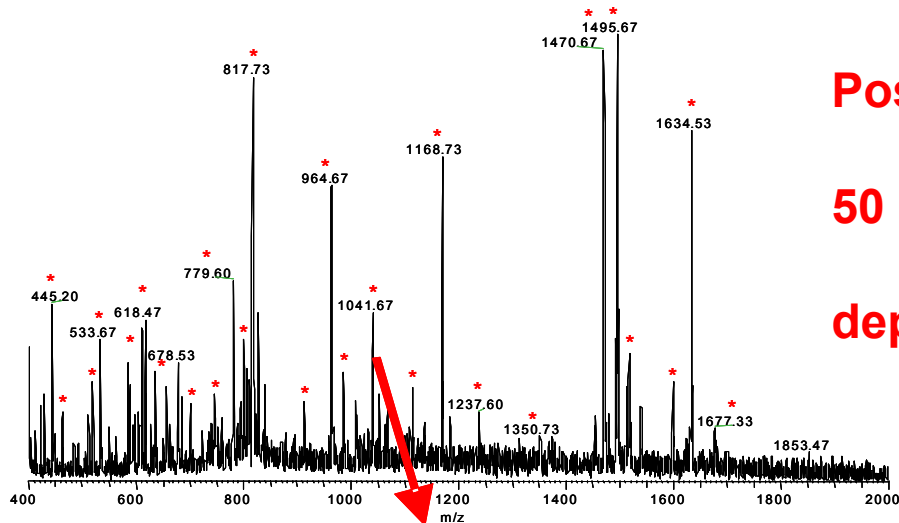
# Peptides, proteins, and bacteria

$M^*$

- Ambient samples; 1-10 ng/cm<sup>2</sup>; PTFE surface;
- Methanol/water spray; 5 sec analysis time

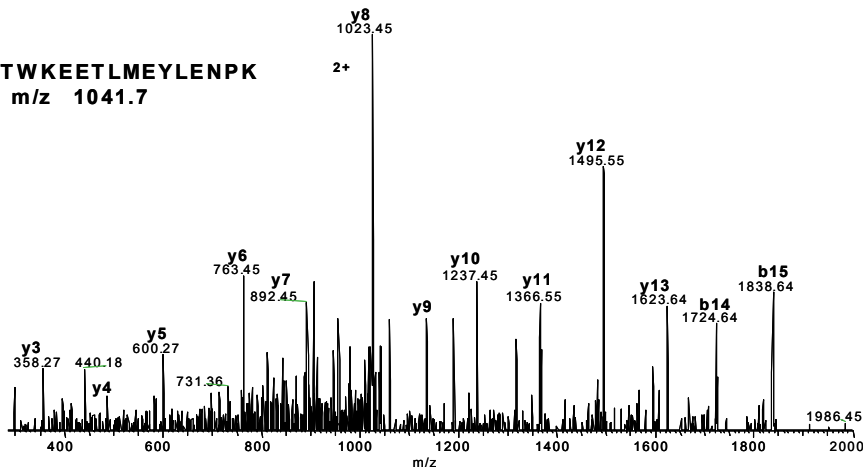


# Analysis of tryptic digest by DESI



**Positive ion DESI mass spectrum of  
50 ng equine cytochrome c tryptic digest  
deposited on a non-conducting surface**

**GITWKEETLMEYLENPK  
at m/z 1041.7**



**DESI-MS/MS of m/z 1041.7**

**~97% sequence coverage**

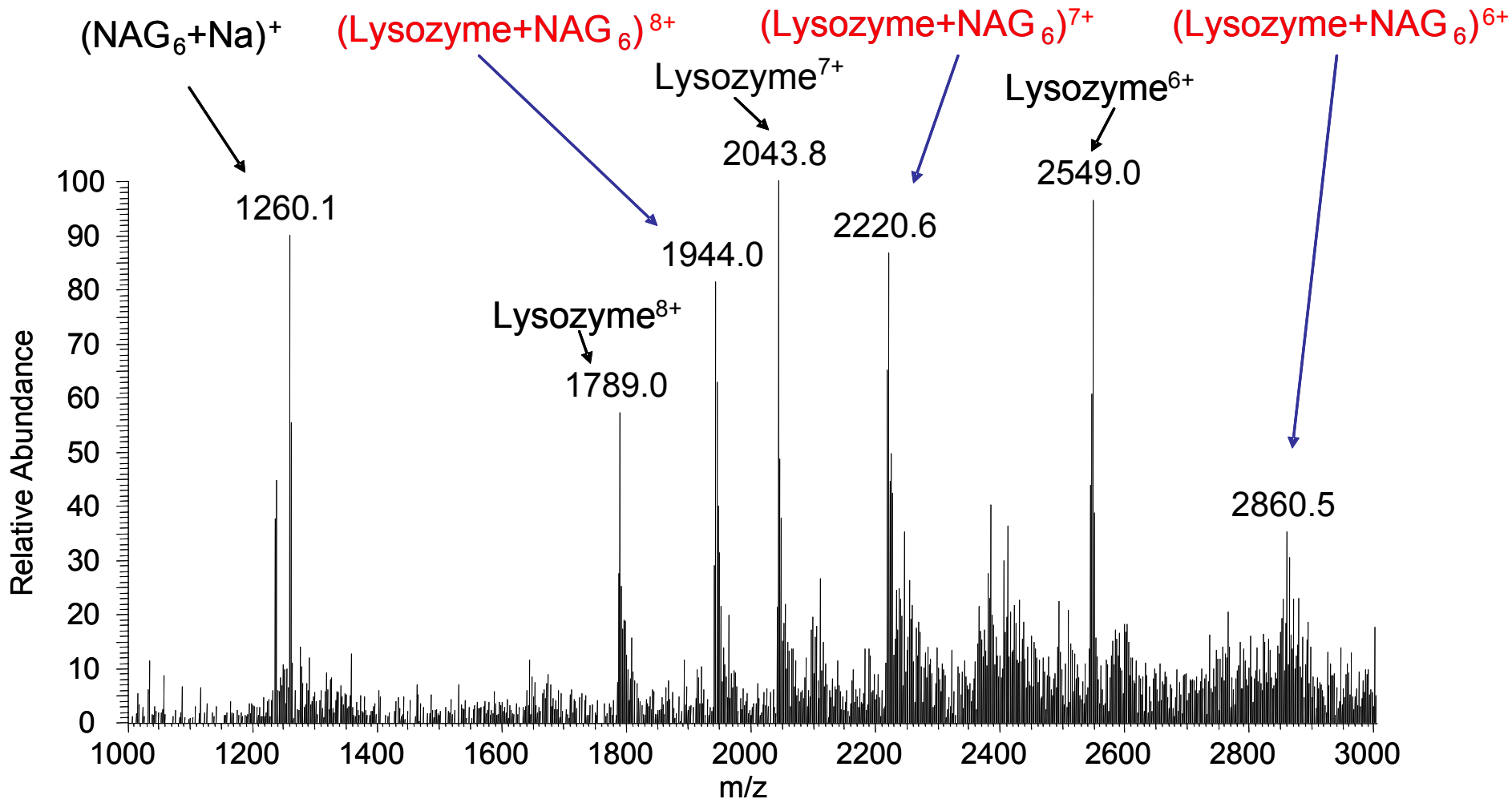
# Reactive DESI

## Ligand (G) + Analyte (S) $\rightarrow$ Ligand-Analyte (GS)

- Spray carries reactant species to the surface
- Detect reaction products or their CID fragments
- Covalent reaction products
  - methylation, hydroxymethylation
- Non-covalent complexes – enzyme/substrate, amino acid cluster, etc.
- Examples
  - Use of chiral selectors in spray for chiral surface analysis
    - Diastereomeric, metal centered complexes
    - Serine/threonine
  - Formation of proton-bound dimers
  - Ligand protein complexes for fishing

# Reactive Desorption – Lysozyme and Hexa-N-acetyl chitohexaose

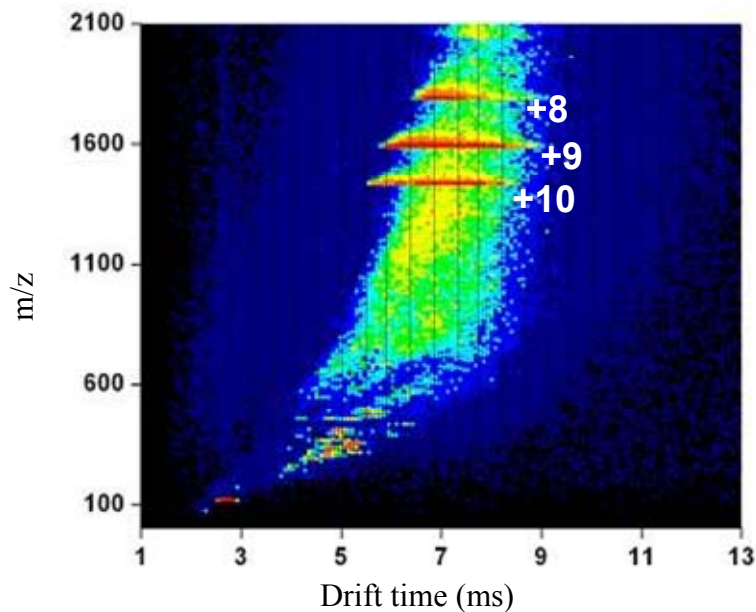
$\mathcal{M}^*$



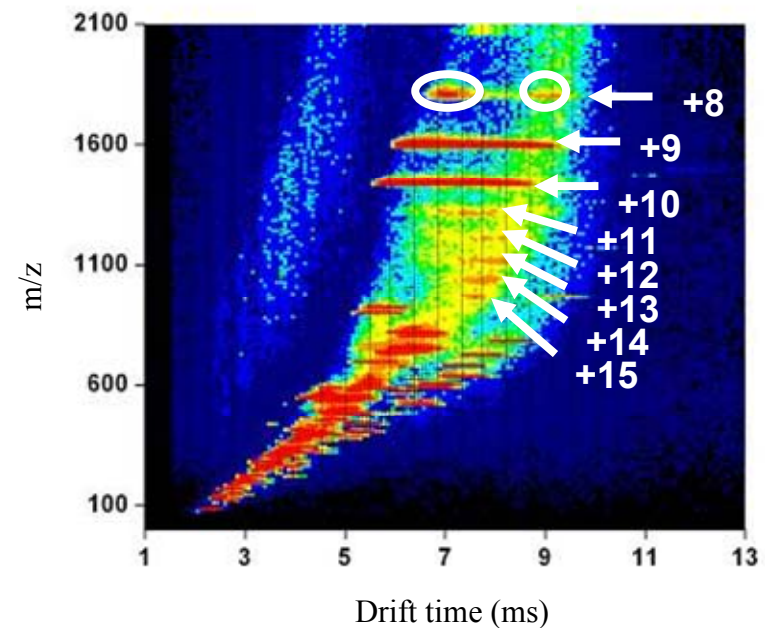
# DESI Ion Mobility TOF: Comparing $M^*$ lysozyme with DESI and ESI

- DESI analysis: Lysozyme was prepared in water, deposited onto PMMA and allowed to dry prior to analysis. 50% methanol:water was used in the spray.
- ESI: Lysozyme was sprayed directly from a water solution.

## DESI-IMS-MS



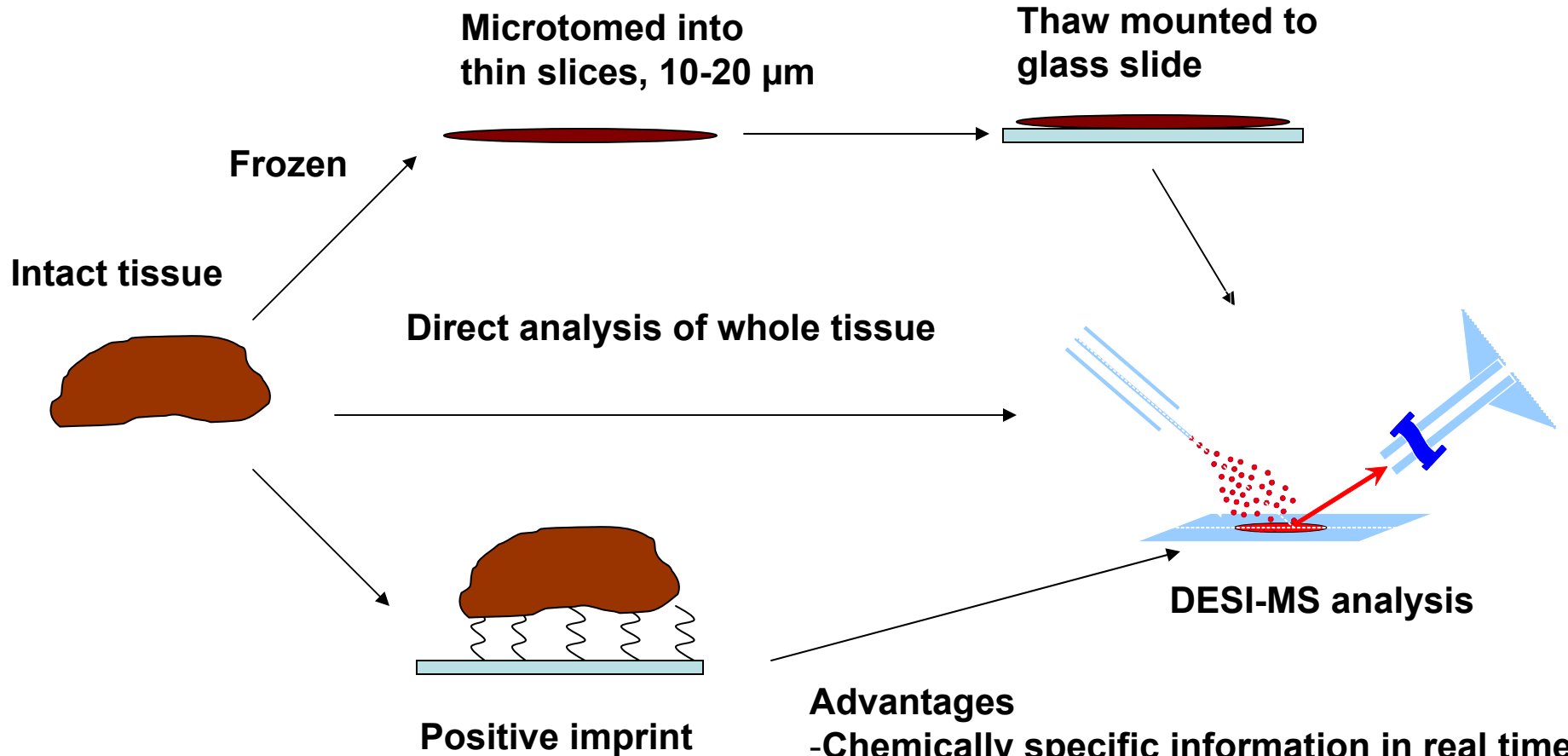
## ESI-IMS-MS



Two distributions are observed for the 8+ charge state in ESI while only 1 is observed in DESI

# Tissue analysis

$M^*$



**DESI-MS analysis**

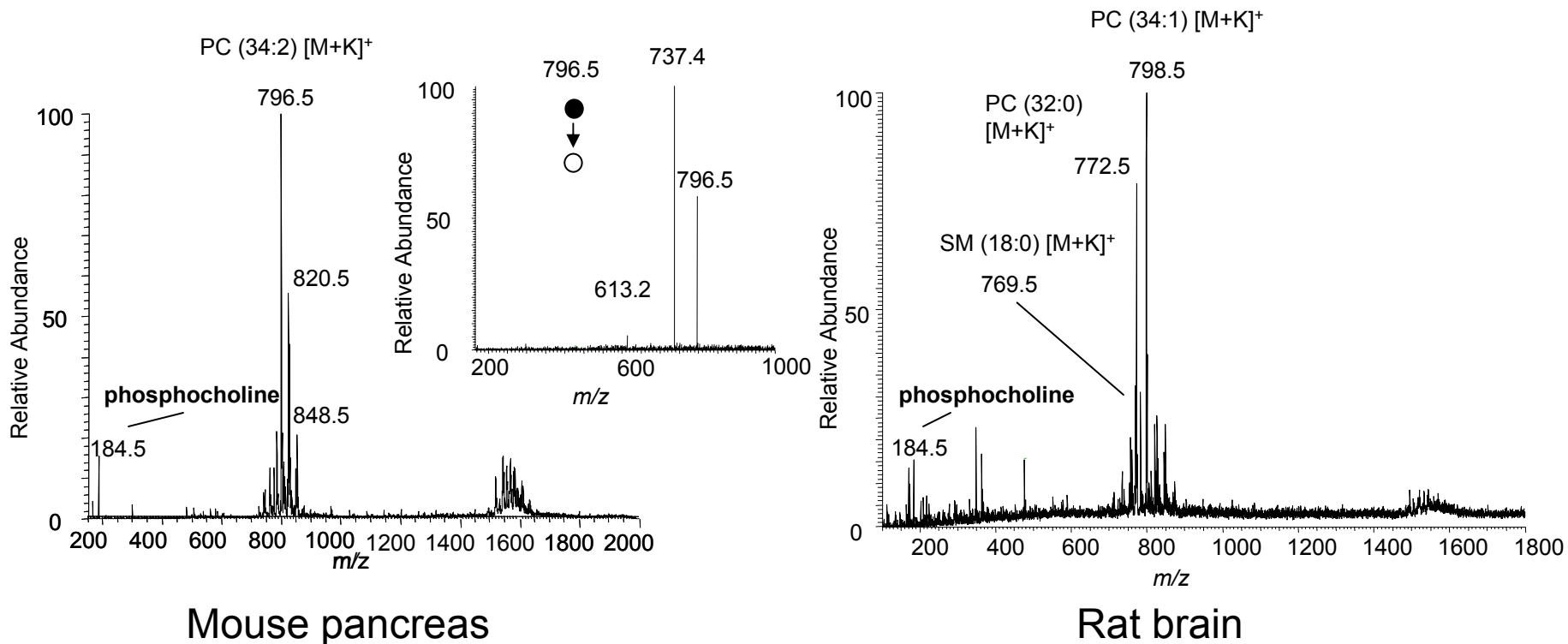
## Advantages

- Chemically specific information in real time
- Works under ambient conditions in atm. for access to the sample
- No sample treatment

# Analysis of mouse pancreas and rat brain tissue

*M\**

Sectioned tissue thaw mounted onto a glass slide



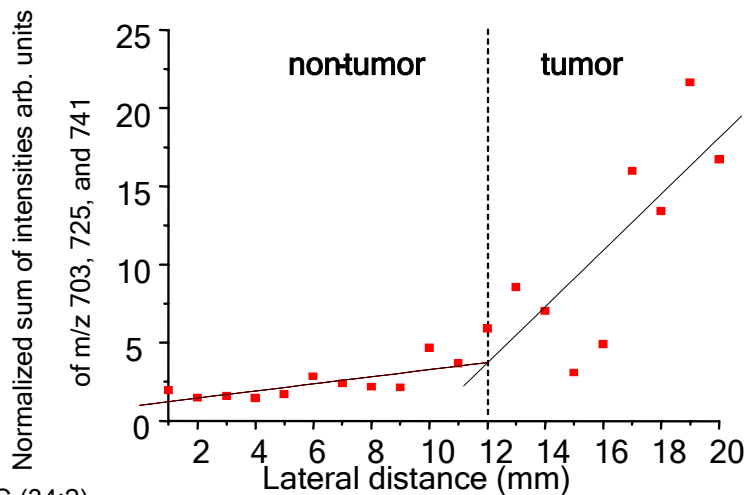
Mouse pancreas

Rat brain

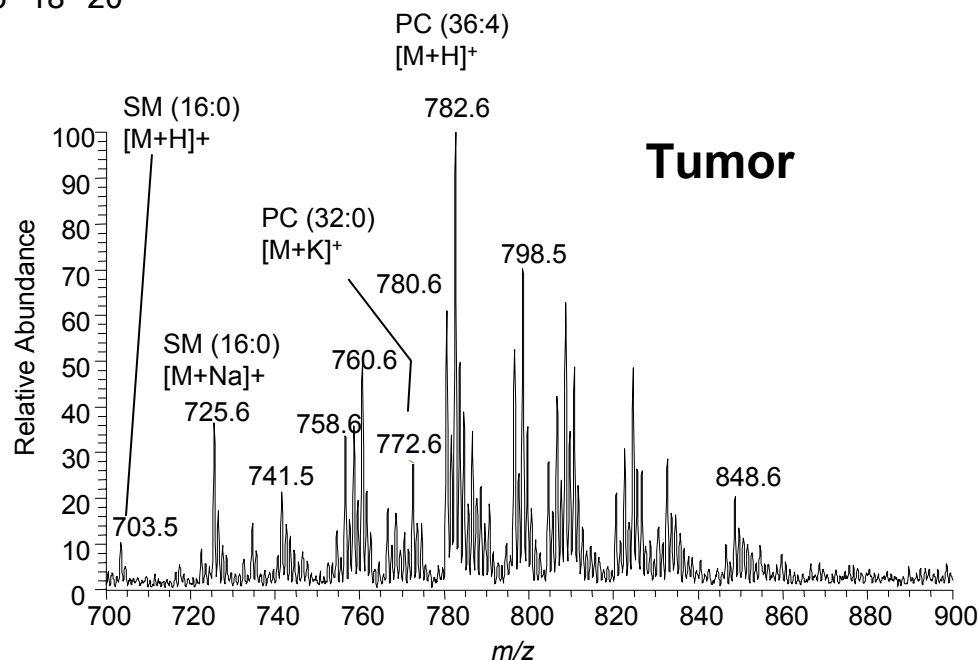
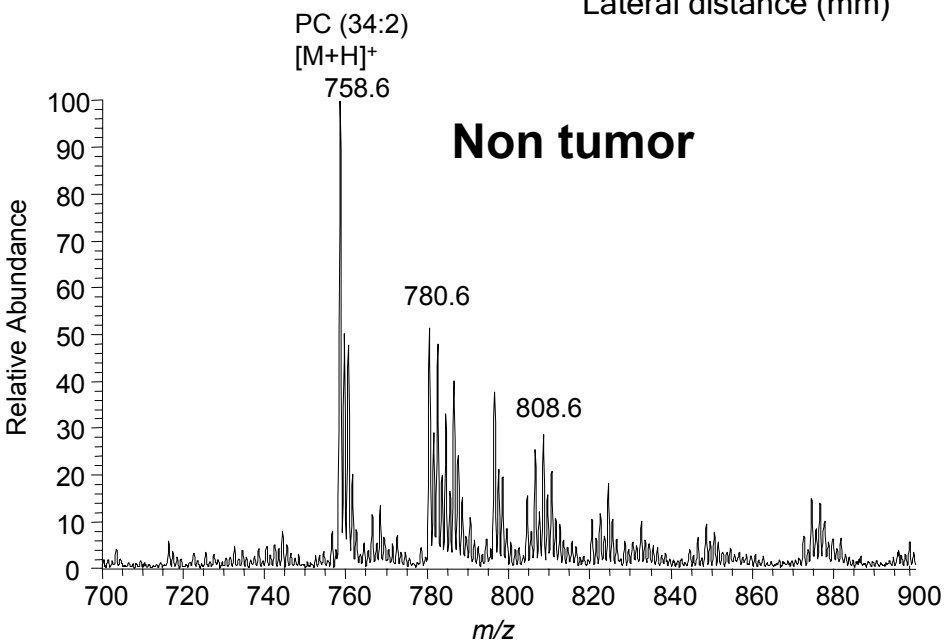
- Positive ion mode
- Methanol/water/acetic acid spray
- 3  $\mu\text{L}/\text{min}$  at 7 bar  $\text{N}_2$  pressure

# Chemical profiling in human liver adenocarcinoma tissue

$M^*$



- Spot sizes are < 1mm
- No sample treatment after microtoming
- Tumor margins can be identified
- Chemically specific information can be obtained directly from the tissue

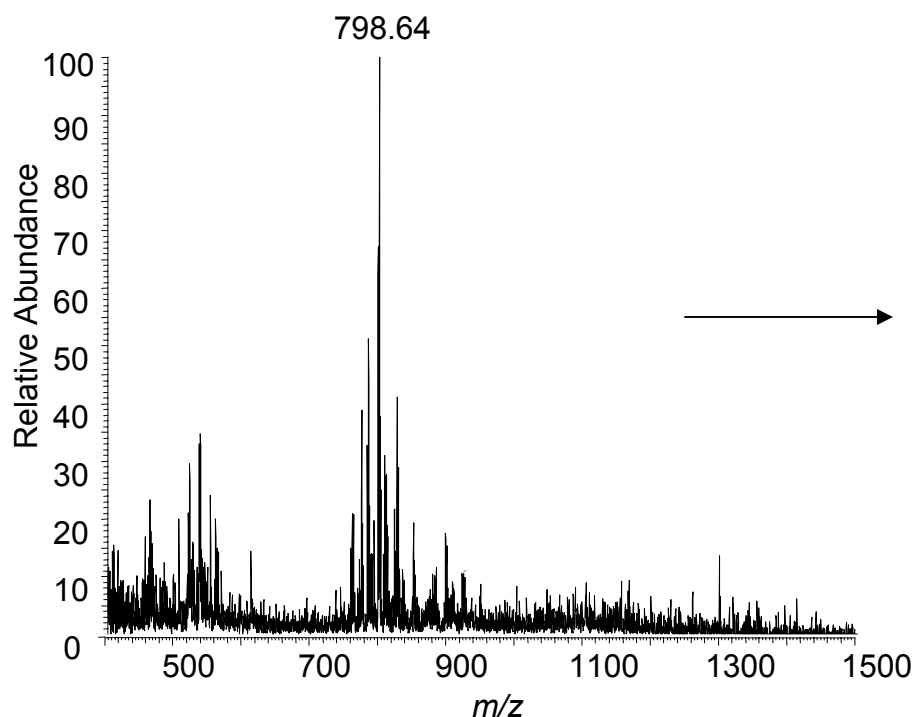


# Intact tissue analysis – heart tissue

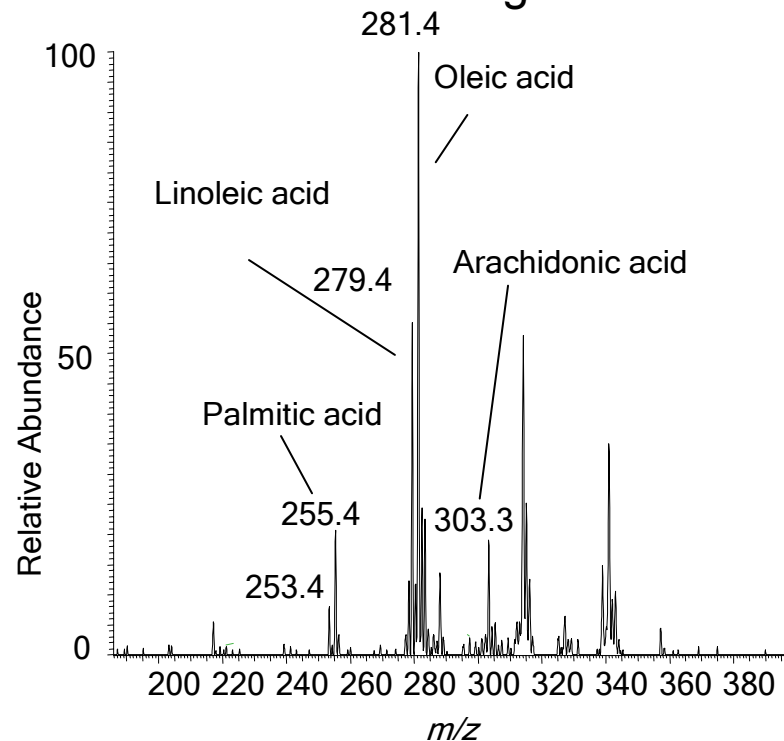
*M\**

Direct analysis of intact chicken heart

Positive ion mass spectrum



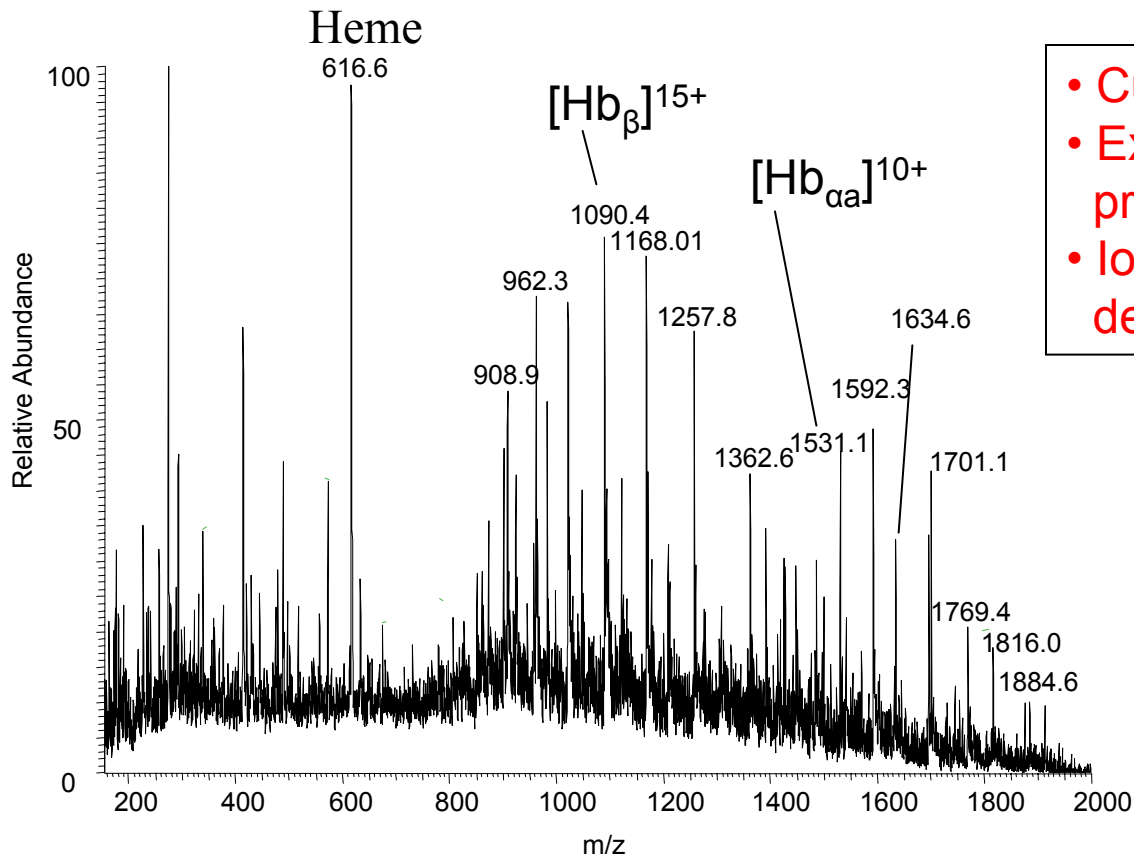
Negative ion spectrum in low mass range



• Methanol/water/ spray

# Direct analysis heart tissue

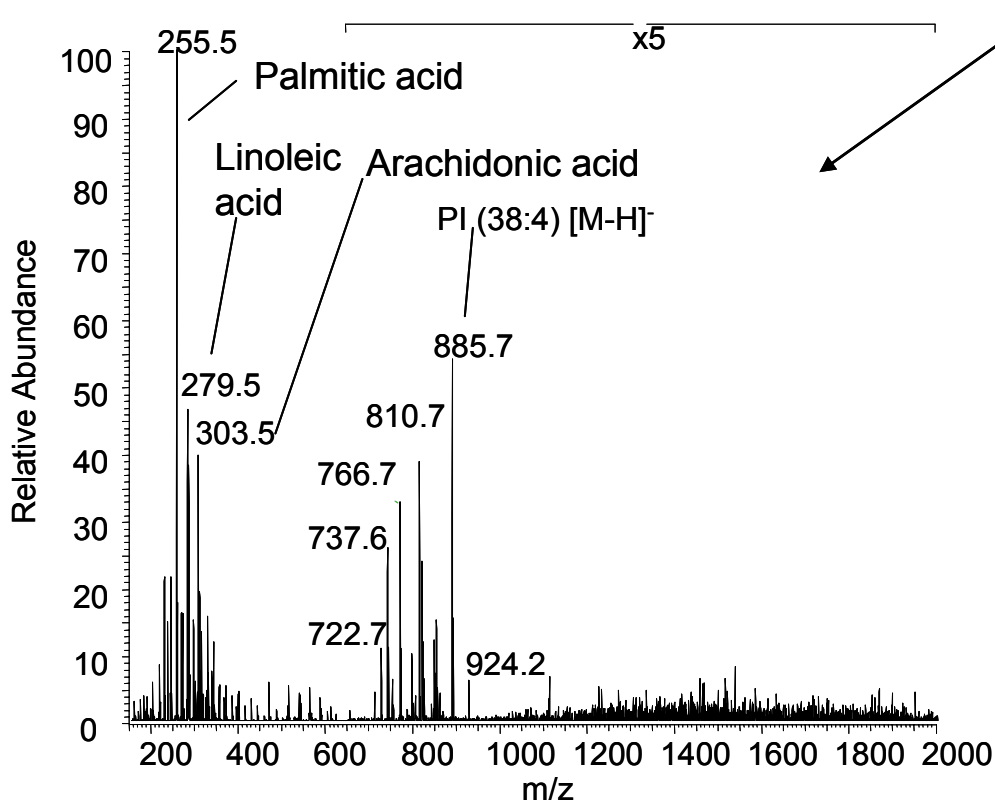
*M\**



- Cut gallus heart tissue
- Exposed directly to the DESI probe
- Ions from hemoglobin chains detected

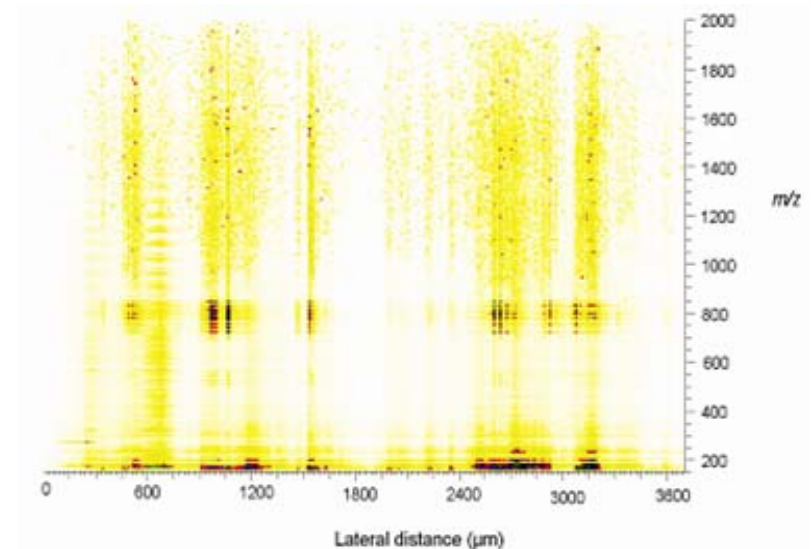
# Rat kidney scan

*M\**



- Negative ion DESI mass spectrum of a rat kidney cross section
- Preliminary chemical assignments show the presence of phosphatidylinositols
- Free fatty acids are also detected

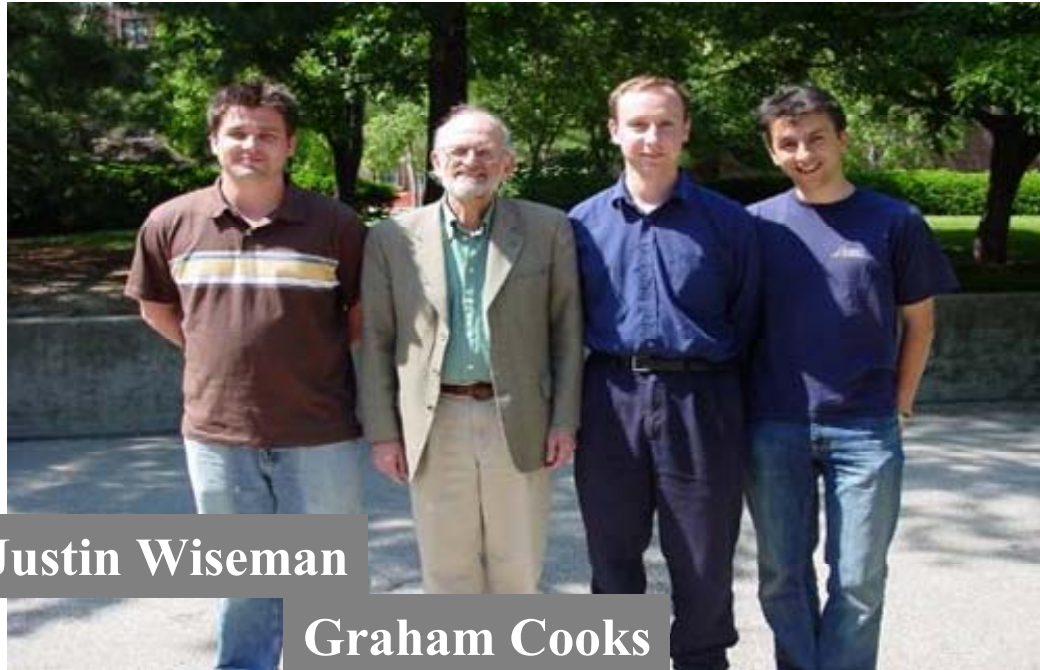
## Total ion map of rat kidney Cross section



# Conclusions

- New technique for sampling surfaces
- Wide applicability in many areas of chemistry and biology
- Technique works best for non-conducting surfaces
- Demonstrated chiral quantification from analytes present on surface (cotton swab)
- Very low levels chemical explosives can be detected on natural surfaces
- Salt tolerance is high relative to APCI for metabolite detection in urine
- Very complex matrices can be analyzed directly such as mammalian tissues and urine – although discrimination effects are apparent

# Acknowledgments



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