

# Liquid Extraction Surface Analysis (LESA™) Combined with Automated nESI-MS/MS as a Novel Tool in the Bioanalytical Laboratory

- Authors: [Daniel Eikel](#)<sup>1</sup> and Yoshiharu Naito<sup>2</sup>
- <sup>1</sup>Advion BioSystems, Inc., Ithaca, NY, USA
- <sup>2</sup>Advion Japan KK, Tokyo, Japan

## Introduction

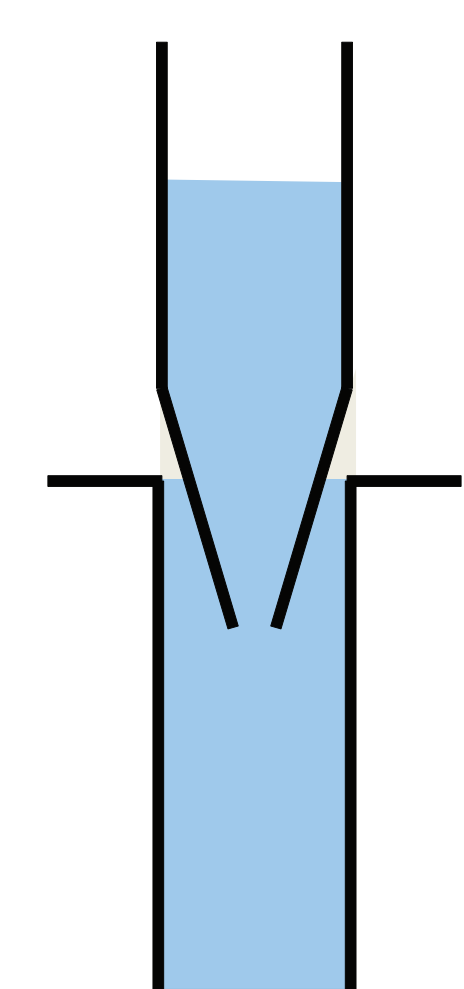
- Liquid Extraction Surface Analysis (LESA) is a novel surface analysis technique based on automated, online solvent extraction and nESI-MS (1)**
- Here we present LESA applied to two bioanalytical challenges: Dried Blood Spot (DBS) analysis and small molecule profiling from thin tissue sections**



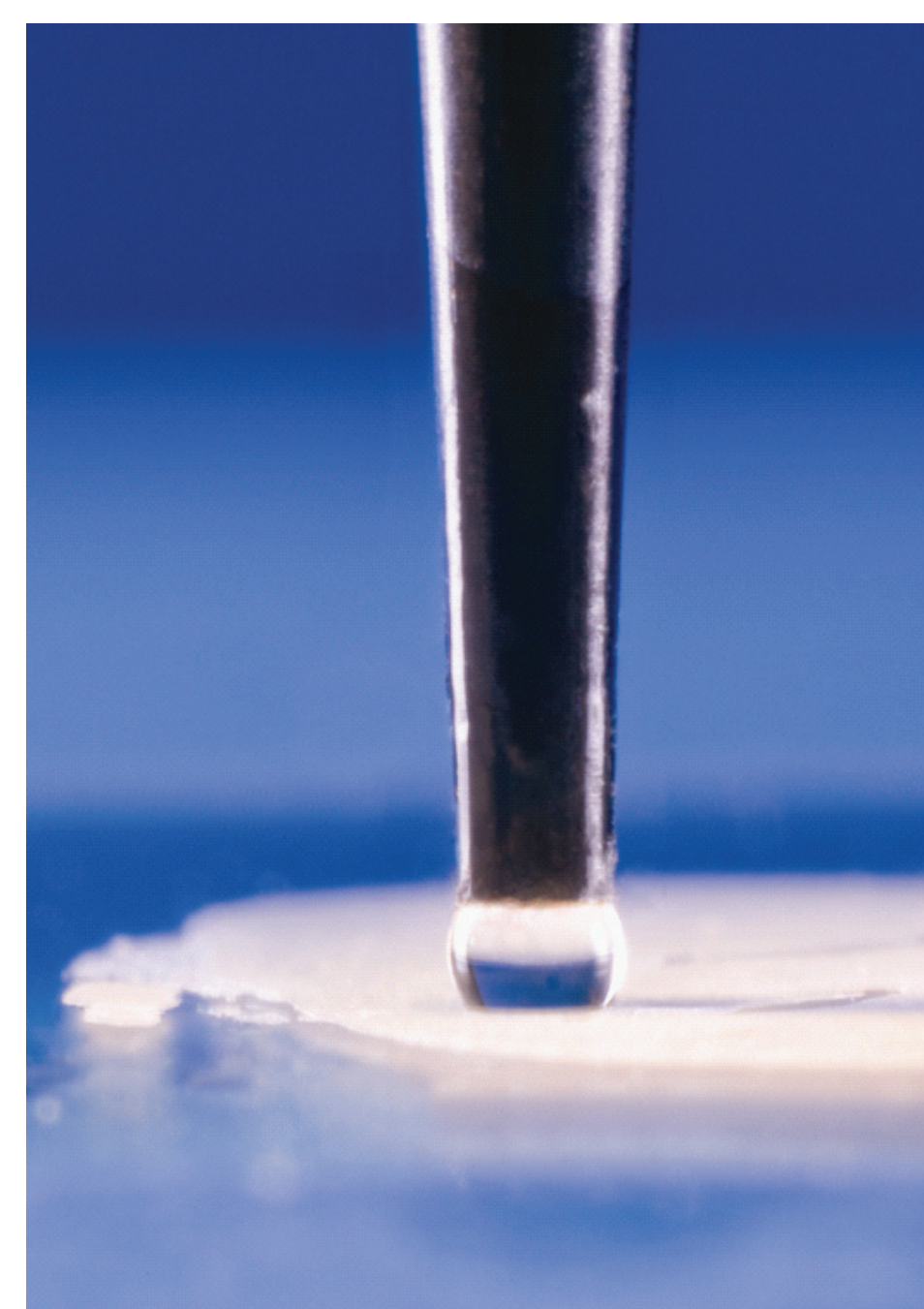
TriVersa NanoMate® robotic nanoESI source from Advion BioSystems, Inc., Ithaca, NY USA

## LESA Schematic Workflow

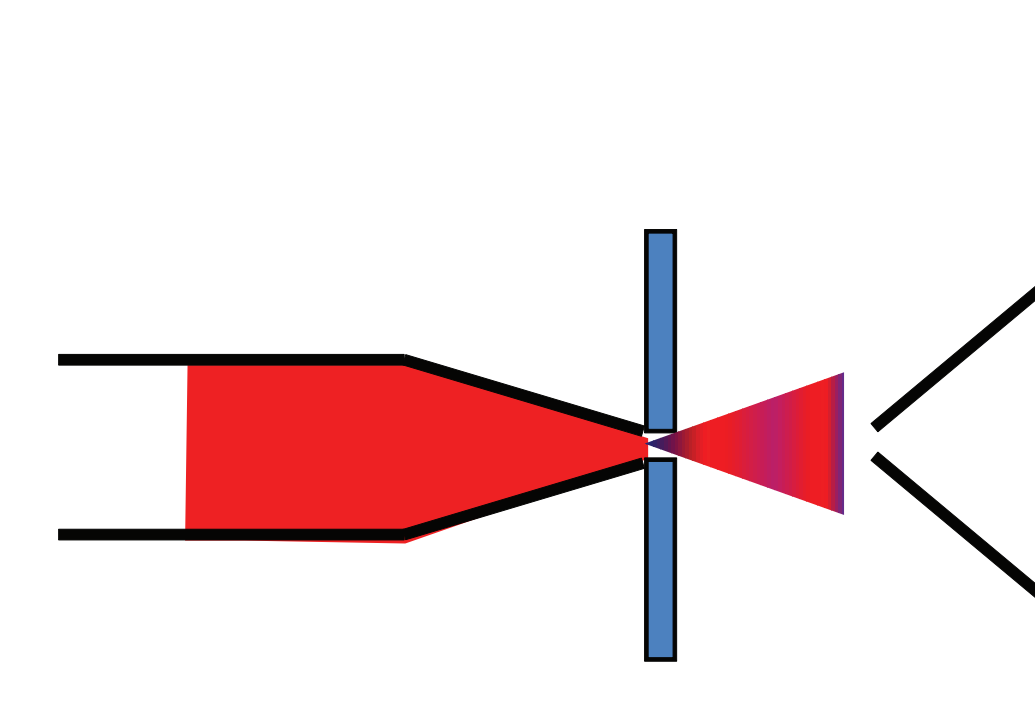
**Step 1: Solvent Delivery**  
Disposable tip picks up extraction solvent at reservoir



**Step 2: Analyte Extraction**  
Robot places extraction solvent on target and initiates aspirate/dispense cycles for analyte extraction



**Step 3: Analyte Ionization**  
Robot aspirates extracted analytes from target and initiates electrospray at a 400-nozzle nanoESI chip

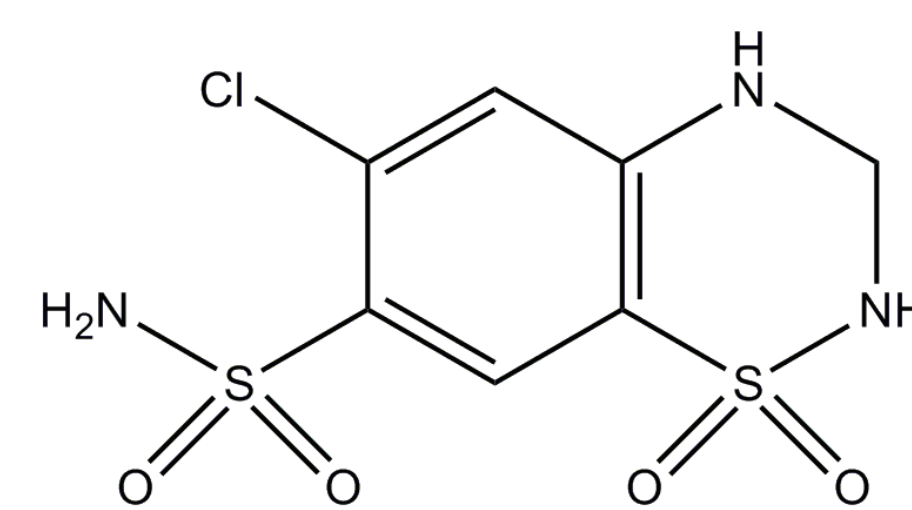


## LESA of Small Drug Molecules from Dried Blood Spots



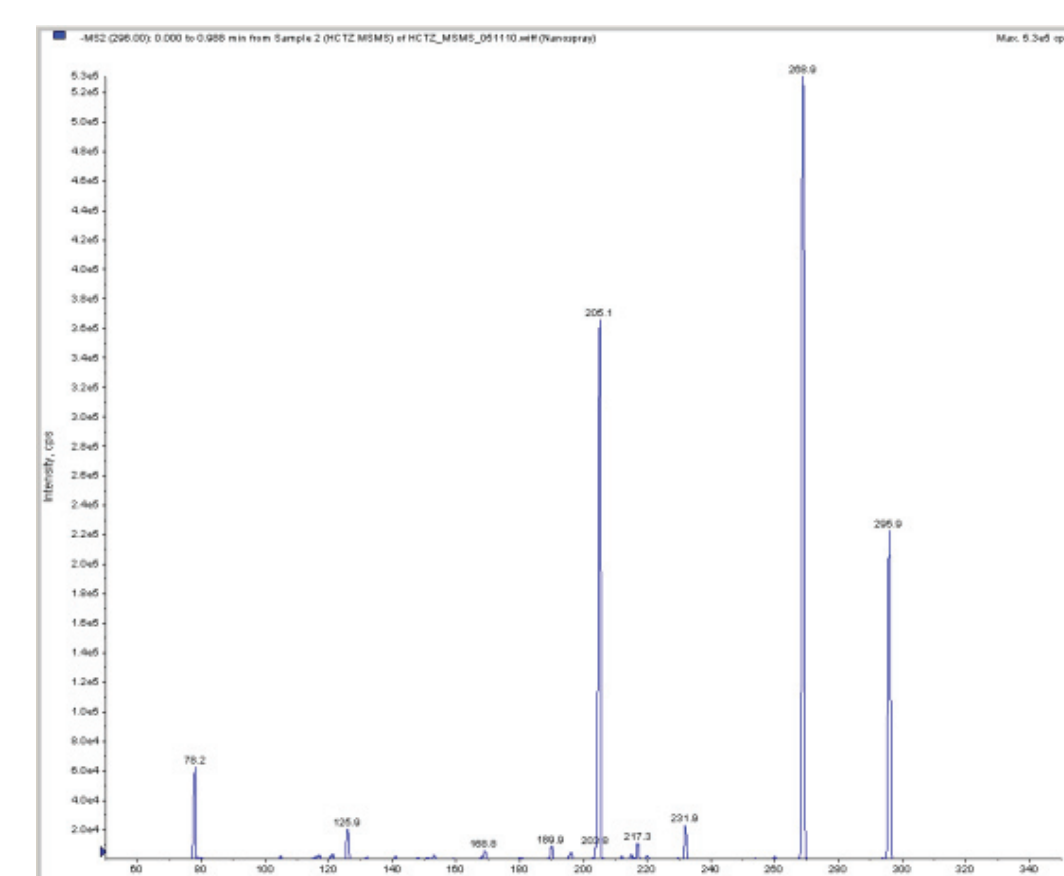
LESA analysis from Dried Blood Spots

Hydrochlorothiazide (HCTZ) is a diuretic drug of the thiazide class.



Hydrochlorothiazide

Chemical Formula: C<sub>7</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>4</sub>S<sub>2</sub>  
Exact Mass: 296.96

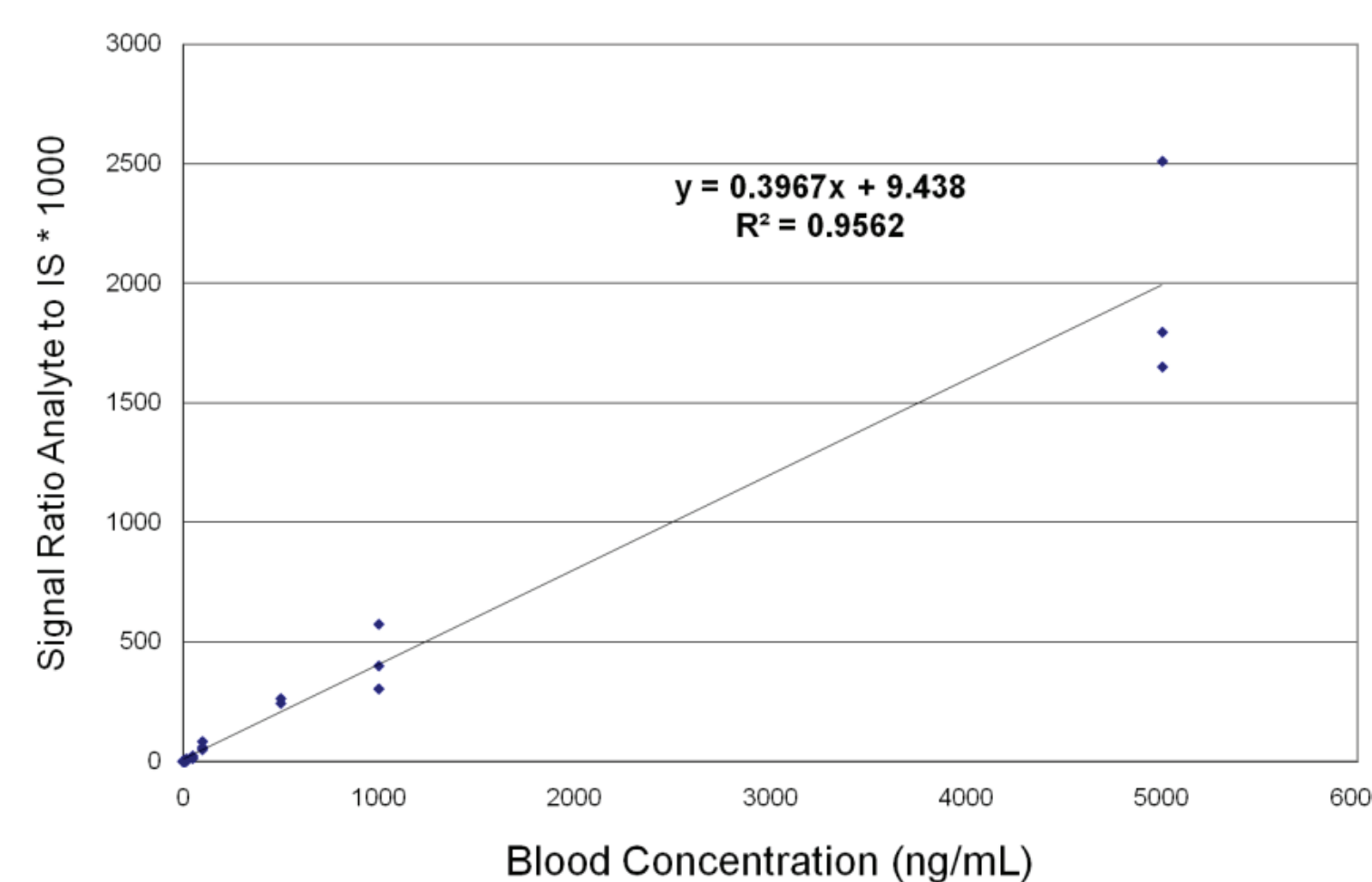


HCTZ MS/MS in negative ion mode

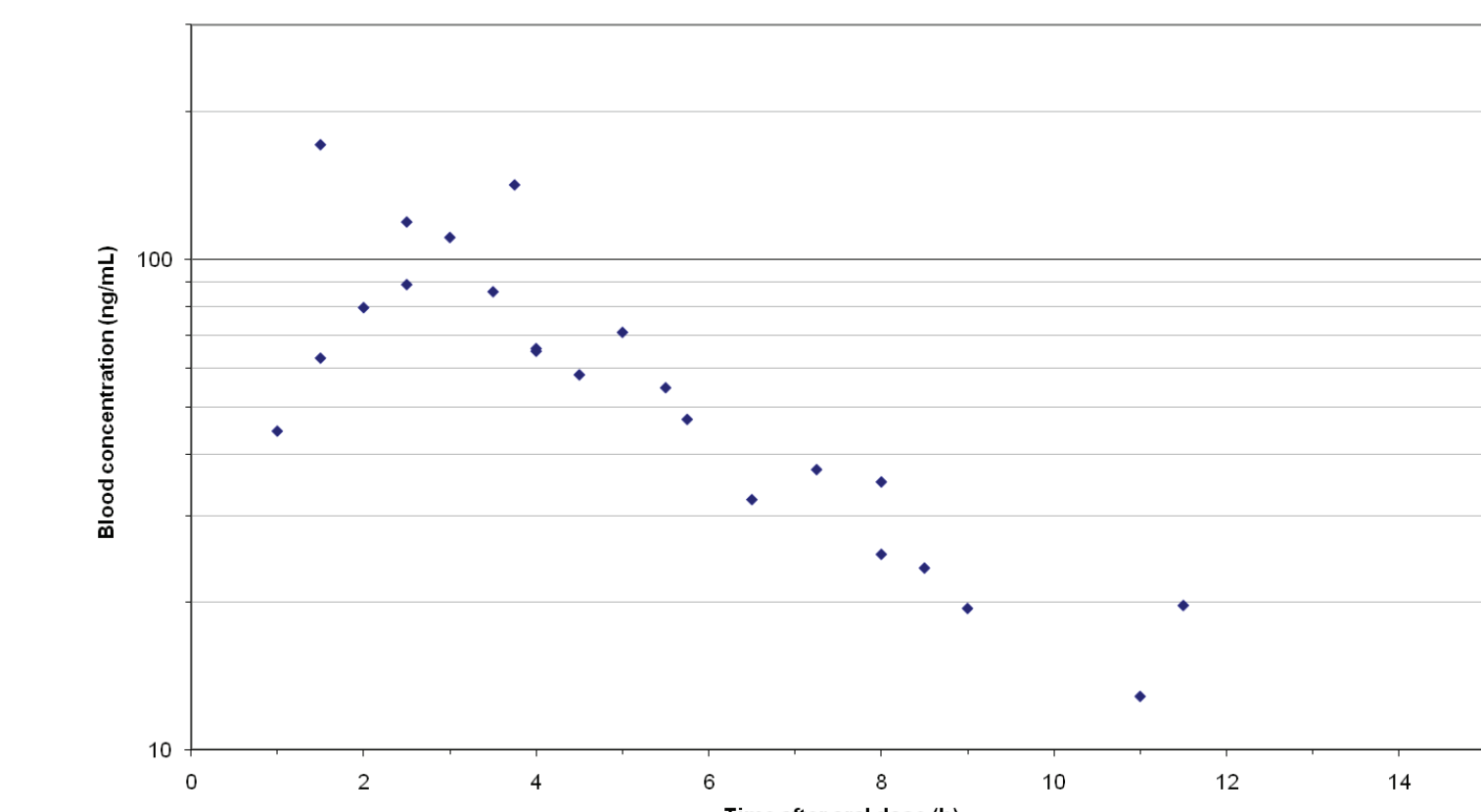
**Method** 12 µL fortified lithium heparin blood (Bioreclamation, Inc., USA) was spotted onto Ahlstrom 237 filter paper and dried for 2 hours at room temperature (standard curve range: 10-5,000 ng/mL). DBS samples were mounted on a 96-well plate via tape and spray-coated/soaked with silicone (Dead Down Wind, LLC, MO, USA) and Kiwi Camp Dry spray (Sara Lee Household and Body Care, PA, USA) followed by drying for 1 hour at room temperature (2). Analyte was then extracted using 80/20 MeOH/water 0.1 vol% NH<sub>4</sub>OH with 50 ng/mL HCTZ-IS (position 13 carries <sup>13</sup>CD<sub>2</sub>) and electrosprayed at ca. 300 nL/min, negative ion mode detection at 1.3 kV using SRM for HCTZ (295.9/268.9) and its stable label internal standard (298.9/271.9).

## HCTZ Direct LESA DBS Analysis

Standard curve for HCTZ  
(9 calibrants covering 10 – 5000 ng/mL, S/N of 5 @ 10 ng/mL)

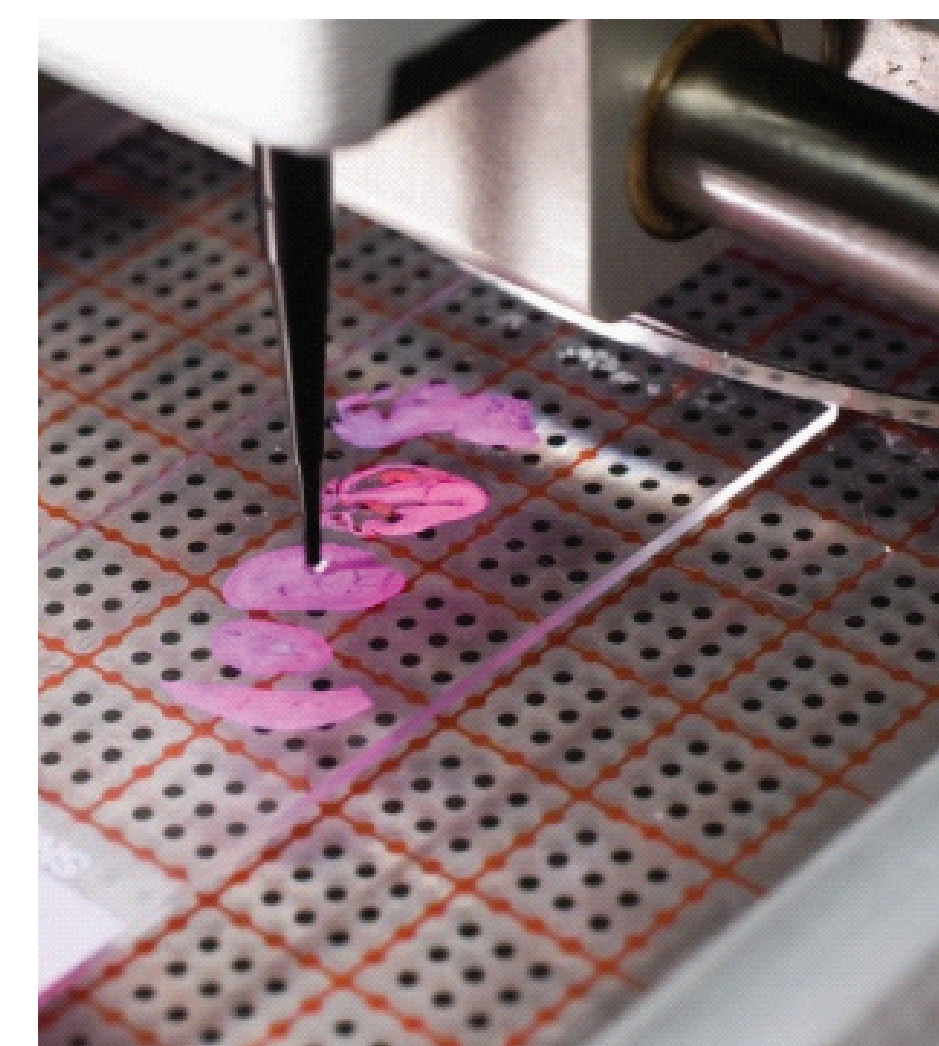


HCTZ pharmacokinetic after 0.25 mg/kg oral dose  
(two volunteers, data points from three consecutive days)



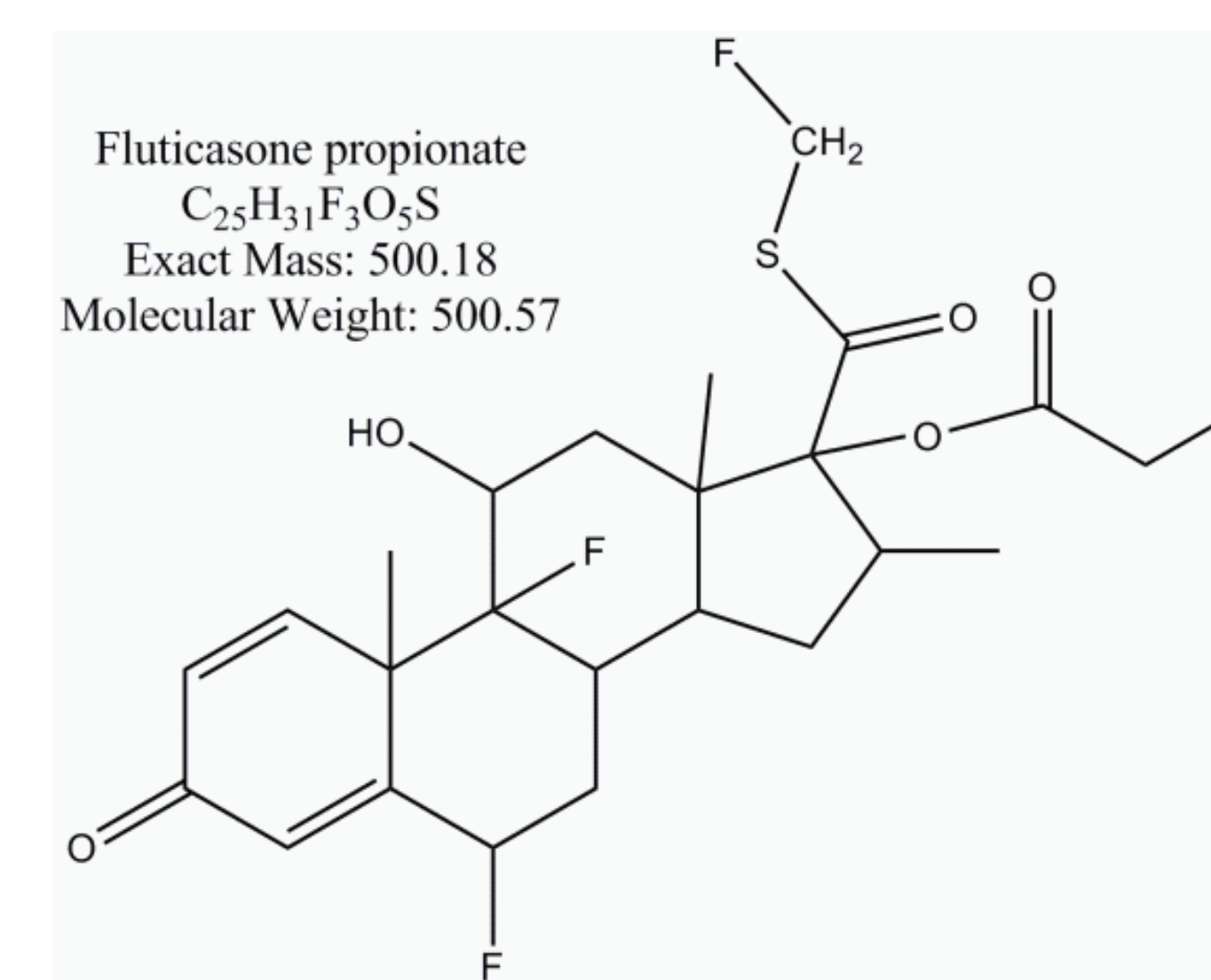
Hydrochlorothiazide (HCTZ) can be directly quantified from silicone-treated DBS media with an LOQ of 10 ng/mL. Method development is rapid due to elimination of off-line extraction method and LC separation.

## LESA Analysis of Tissue Sections



Analysis from whole body slices or single organs

Fluticasone is a synthetic corticosteroid, used in asthma treatment and applied as topical drug!

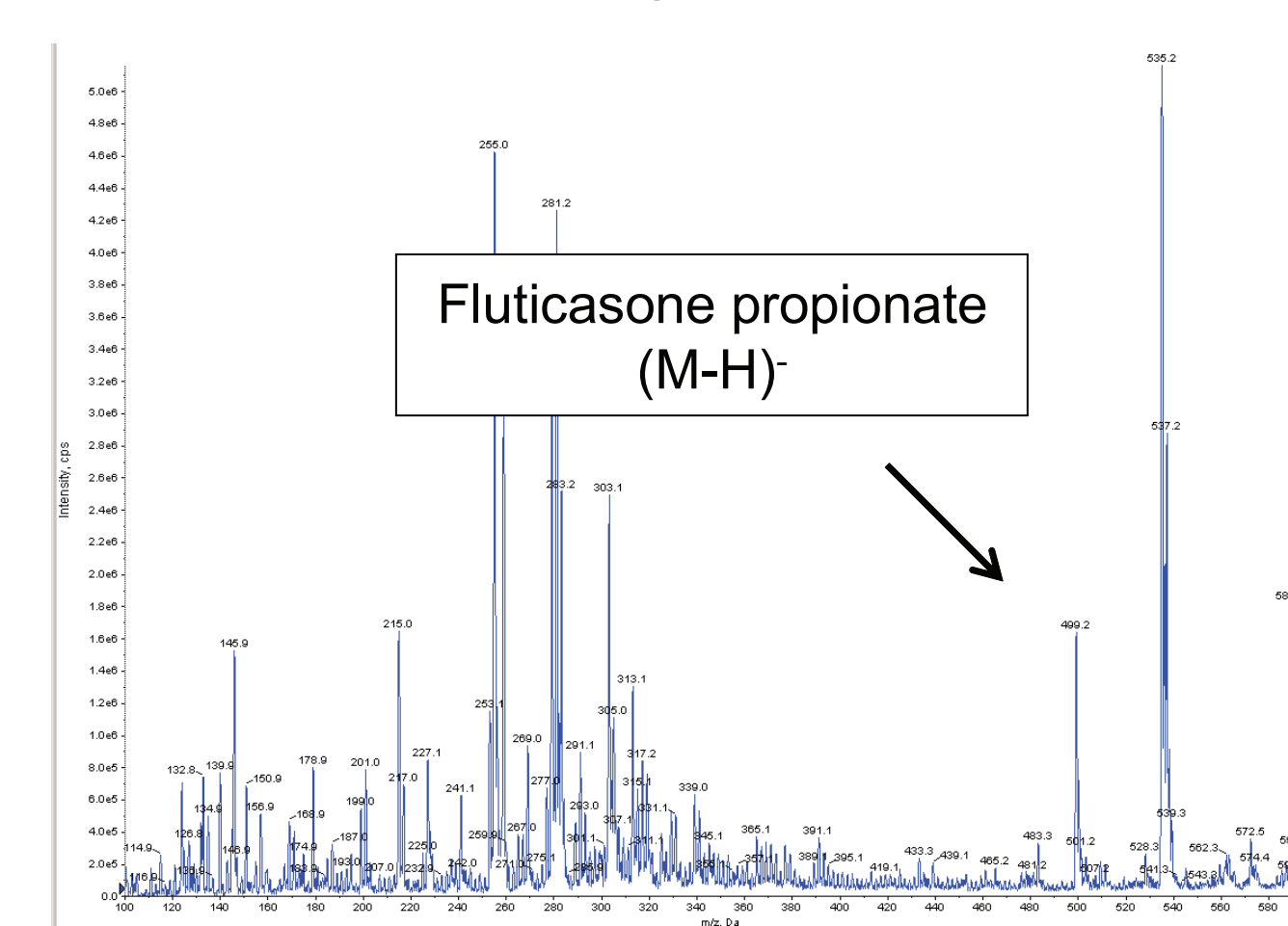


Fluticasone propionate  
C<sub>25</sub>H<sub>31</sub>F<sub>5</sub>O<sub>5</sub>S  
Exact Mass: 500.18  
Molecular Weight: 500.57

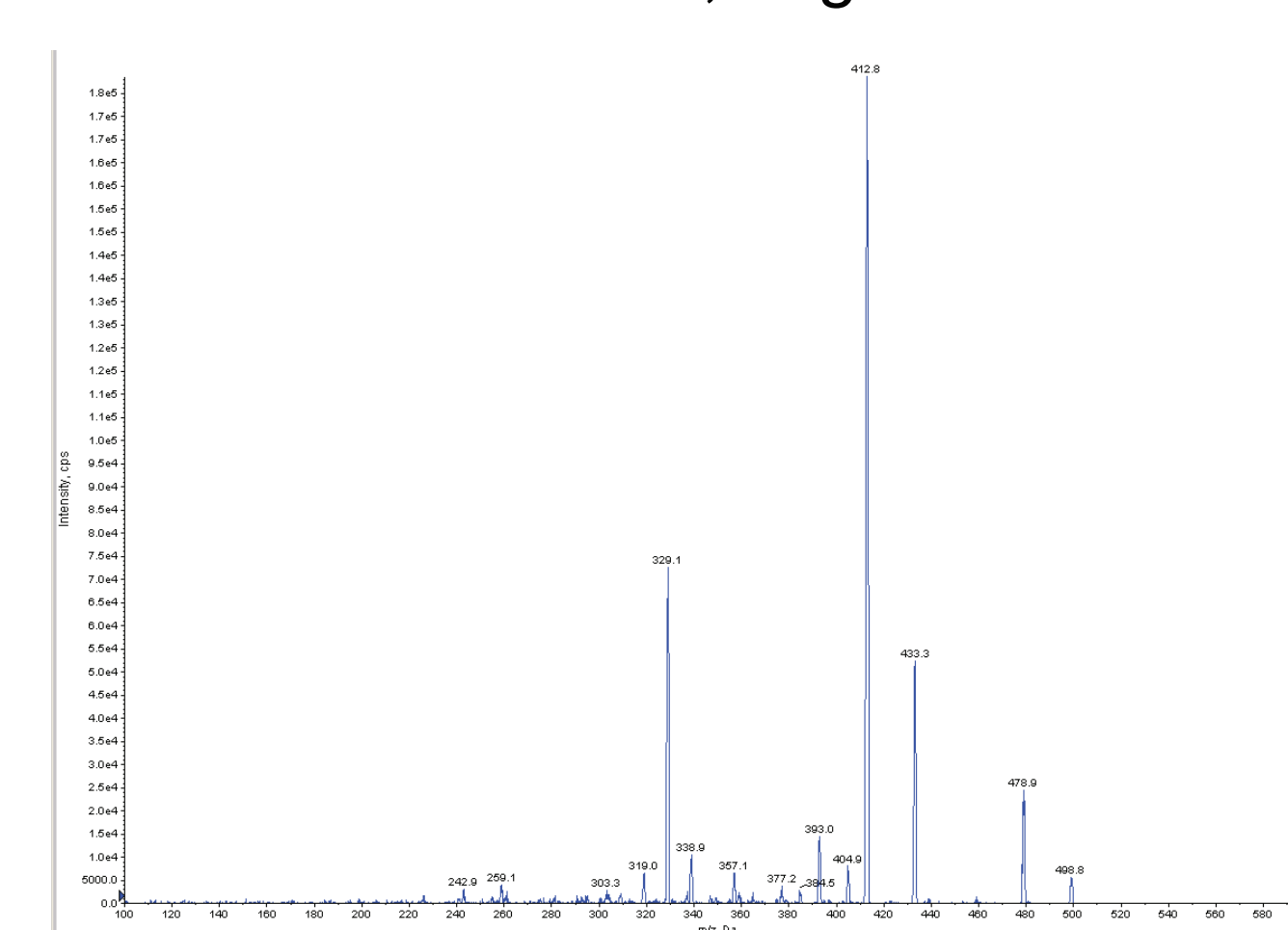
**Method** Whole guinea pig lung is extracted from the animal, connected to a perfusator and mechanically vented before dosed intra-tracheally with 3 mg fluticasone propionate in 5 mL air using a micro-sprayer needle. Tissue was then frozen, sliced in 16 µm thicknesses and placed on glass slides for vacuum drying and further LESA processing. LESA parameters used were 80/20 Methanol/Water 0.1 vol% NH<sub>4</sub>OH as extraction solvent with a setting of 1.5 µL solvent picked up, 1.0 µL dispensed, and 1.1 µL aspirated from the sample surface (asp/disp repeated twice). Analytes were ionized at -1.3 kV in negative ion mode with a pressure assist of 0.9 psi.

## Example LESA-MS/MS Data Obtained from Tissue Sections

Q1 scan, negative ion mode

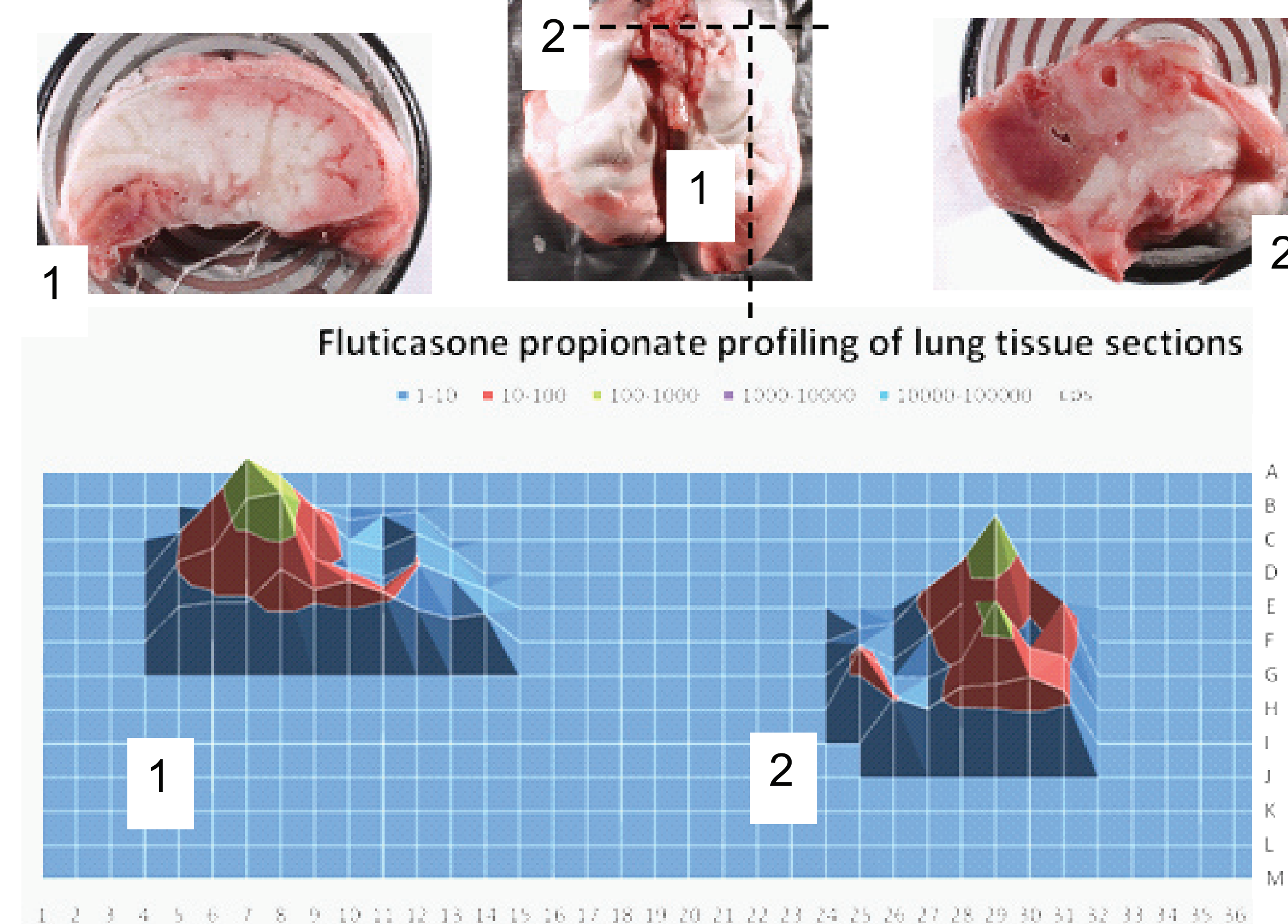


MS/MS of m/z 499.1, negative ion mode



Example data obtained from tissue spot: strong precursor ion signal in negative ion mode and excellent MS/MS data to identify fluticasone propionate.

## LESA-MS Analyte Profiling from Tissue Sections



Surface analysis of fluticasone (SRM transition 499.1/413.0, logarithmic intensity scale) shows spatial distribution of compound in bronchial part of the lung tissue.

## Summary

- Liquid Extraction Surface Analysis (LESA) is a new and versatile tool in the bioanalytical laboratory**
- The TriVersa NanoMate from Advion BioSystems, Inc., is a commercially available instrument for LESA analysis that combines LESA with nanoelectrospray ionization for mass spectrometry**
- LESA allows direct analysis from Dried Blood Spot (DBS) media with good sensitivity and rapid method development times for small molecule quantification (e.g. HCTZ)**
- LESA also provides spatial information for drug distribution studies in tissue sections and allows for drug profiling with a resolution of ca. 1 mm**
- Other areas of use (data not shown) are small molecule analysis from thin layer chromatography (TLC) plates (3) and hemoglobin protein variant analysis from dried blood spots (4)**

## Literature and Acknowledgements

- Kertesz, V. and Van Berkel, G. J.; Fully automated liquid extraction-based surface sampling and ionization using a chip-based robotic nano electrospray platform. *Journal of Mass Spectrometry* 2010 45(3) p252-260.
- Stankovich, J. J.; Walworth, M.J.; Kertesz, V.; King, R. and Van Berkel, G. J.; Liquid Microjunction Surface Sampling Probe Analysis of Dried Blood Spots using an Automated Chip-Based Nano-ESI Infusion Device. 58th ASMS 2010 Salt Lake City, oral presentation in session 'Increasing Throughput for ADME and PK Assays'.
- Henion, J.; Eikel, D.; Rule, G.; Vega, J.; Prosser, S. and Jones, J.; Liquid Extraction Surface Analysis (LESA) of Dried Blood Spot Cards via Chip-Based Nanoelectrospray for Drug and Drug Metabolite Monitoring Studies. 58th ASMS 2010 Salt Lake City, oral presentation in session 'Dried Blood Spot Analysis'.
- Bunch, J. and Cooper, H.; Surface Sampling of Dried Blood Spots for Haemoglobin Variant Analysis by Direct Infusion Chip-Based Nano-electrospray MS and MS/MS. 58th ASMS 2010 Salt Lake City, poster presentation in session 'Ion Sources: ESI'.

The authors would like to thank Walter Korfmaier and Fangbiao Li (Schering-Plough, Merck Research Laboratories, US) for providing the fluticasone-treated guinea pig lung tissues as well as AB SCIEX for the generous loan of a Qtrap 4000 MS system. We also thank Kathy Henion from Advion for poster layout.

This poster was presented July 26th and 27th 2010, at the 8th-annual Japanese HUPO conference in Tokyo, Japan.