Liquid Extraction Surface Analysis Mass Spectrometry (LESA-MS): A Novel Profiling Tool for Drug Distribution and Metabolism Analysis

Daniel Eikel

Advion Inc., Ithaca, NY, 14850, USA

Overview

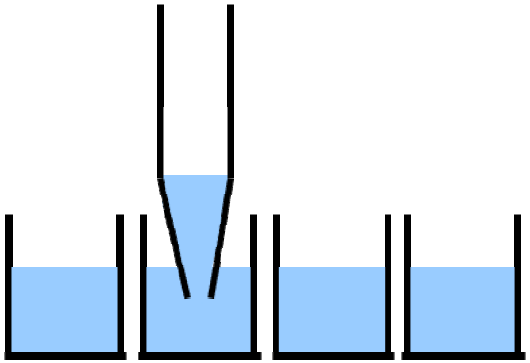
- What is LESA and how does it work ?
- Example 1: Distribution of fluticasone in guinea pig lung tissue
- Example 2: Diclofenac and it’s metabolites
- Example 3: Distribution of terfenadine and its metabolite fexofenadine in whole body sections of mouse
- Summary and Conclusion
- Online Q+A session

What is LESA and how does it work ?

– LESA schematic workflow –

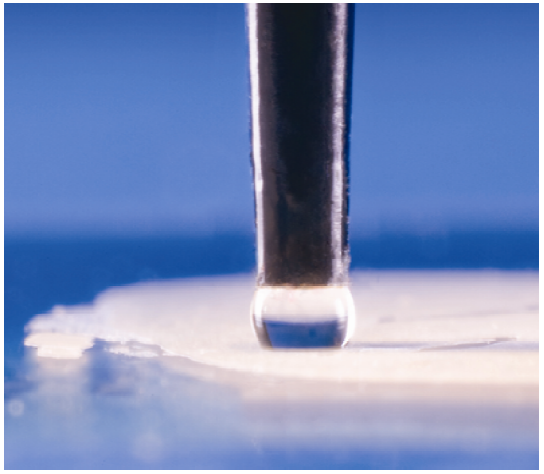
Step 1 – Solvent Selection

Extraction solvent is chosen and a disposable tip picks up the micro liter volume desired from a cooled reservoir



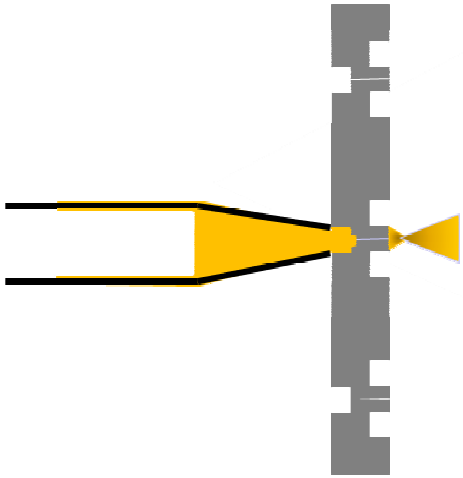
Step 2 – Analyte Extraction

Robot places extraction solvent on the surface, a liquid junction forms and aspirate/dispense cycles are initiated



Step 3 –Ionization

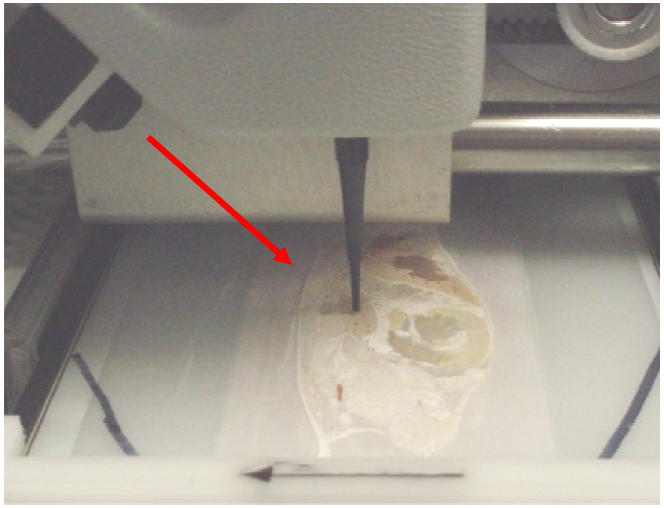
Robot delivers extracted analytes to a 400 nozzle ESI chip and a nano electrospray is generated



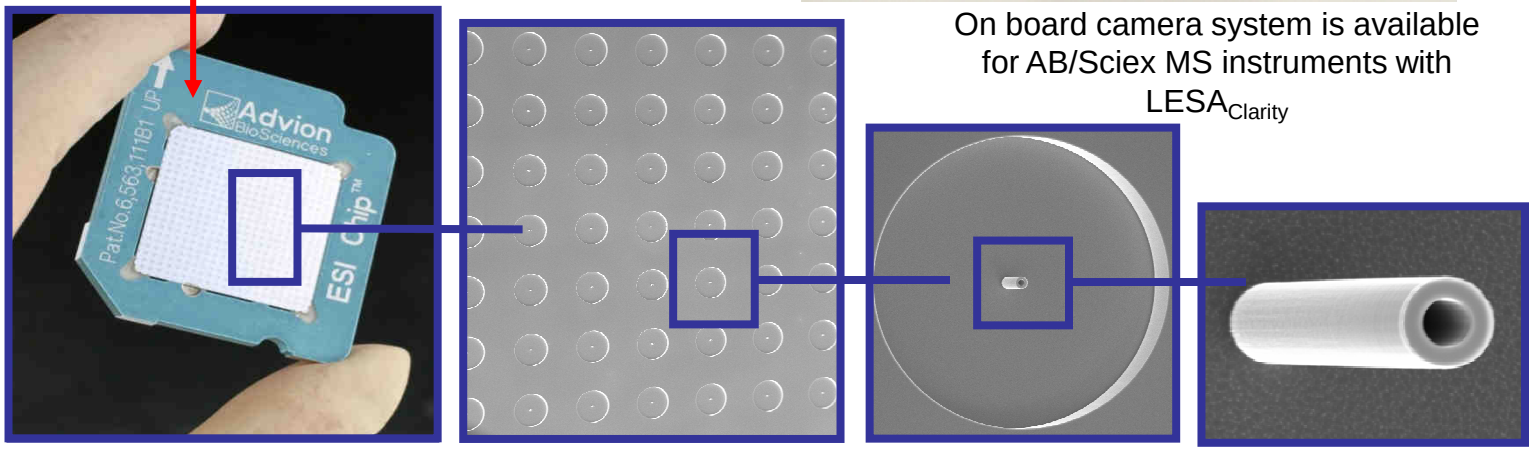
Kertesz V. and Van Berkel GJ: Fully automated liquid extraction-based surface sampling and ionization using a chip-based robotic nano electrospray platform. Journal of Mass Spectrometry **2010** 45(3) 252-260

What is LESA and how does it work ?

– LESA using the Advion TriVersa NanoMate –



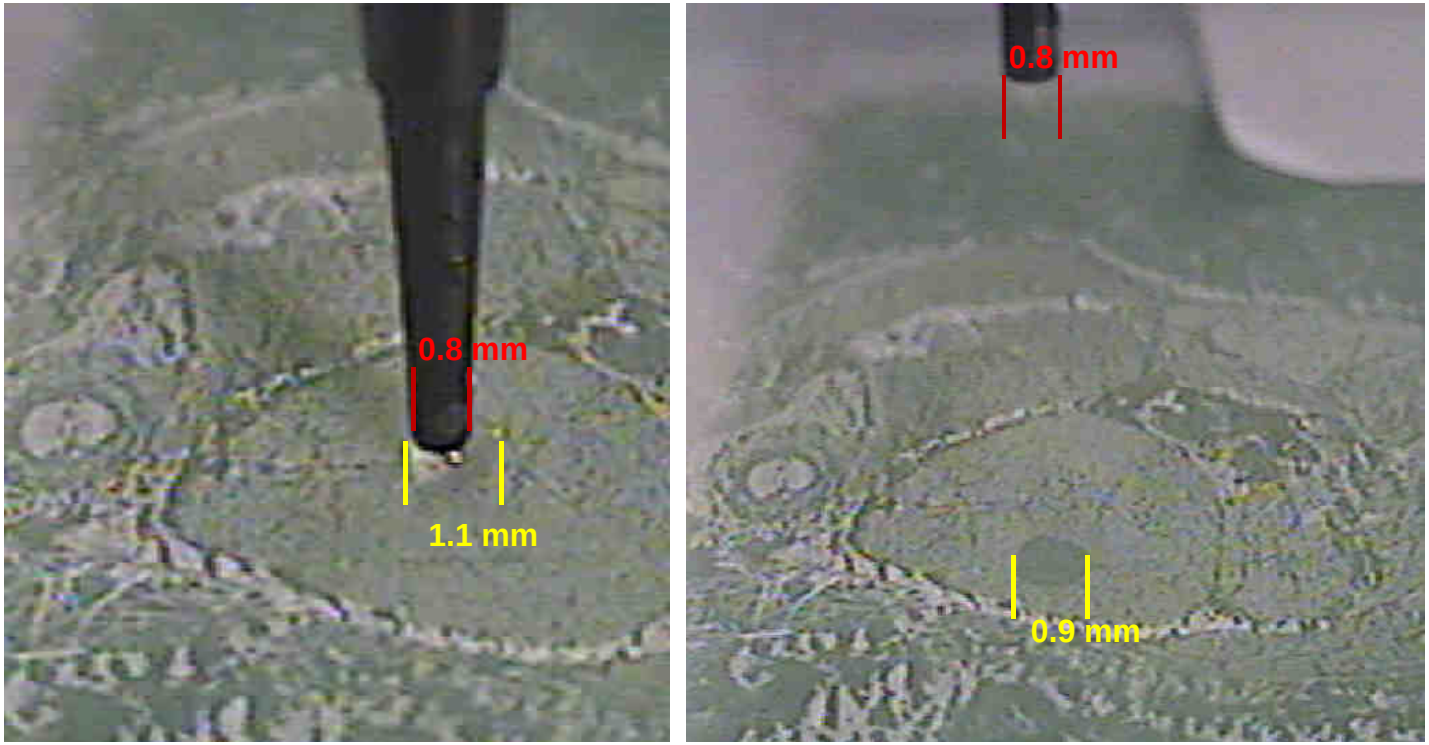
On board camera system is available for AB/Sciex MS instruments with $LESA_{Clarity}$



Nozzle dimensions: 5.5 µm ID x 28 µm OD x 55 µm height

What is LESA and how does it work ?

– Spatial resolution using 80/20 methanol/water 0.1 vol% formic acid –

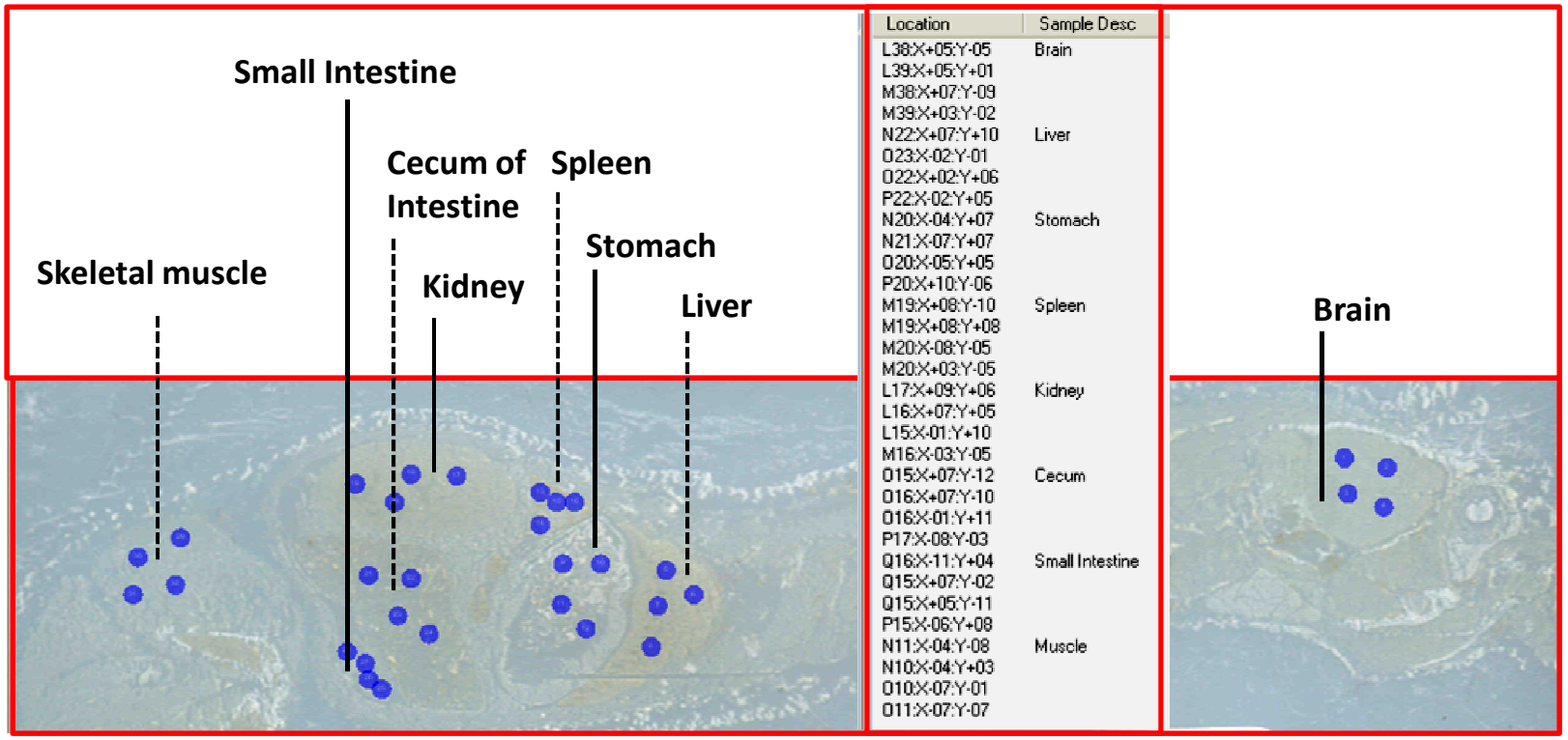


1.2 µL extraction solvent pick up, 0.7 µL dispense on target, 0.9 µL aspirate from target

On board camera system is available for AB/Sciex MS instruments with $LESA_{Clarity}$

What is LESA and how does it work ?

– Sample selection using LESA points software –



Example 1
Distribution of fluticasone in lung tissue

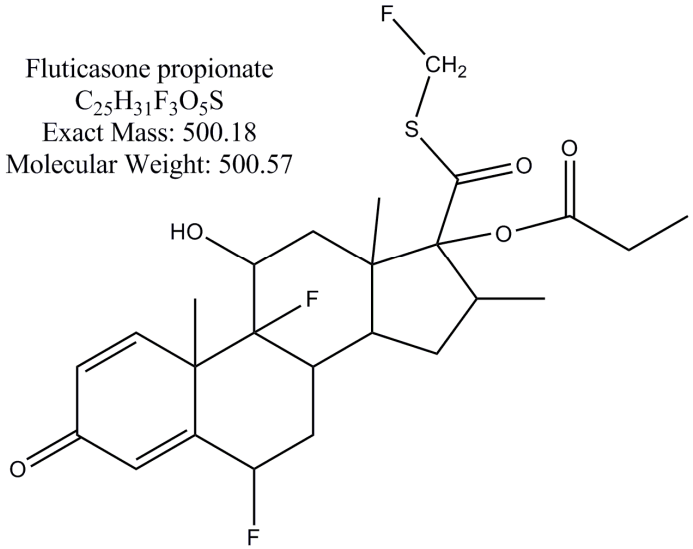
…in cooperation with…

Fangbiao Li and Walter Korfmacher (Merck)
Jack Henion (Advion)
Gary vanBerkel, Vilmos Kertesz, Mathew Walworth and Miriam Elnagger (ORNL)

Example 1: Distribution of fluticasone in lung tissue

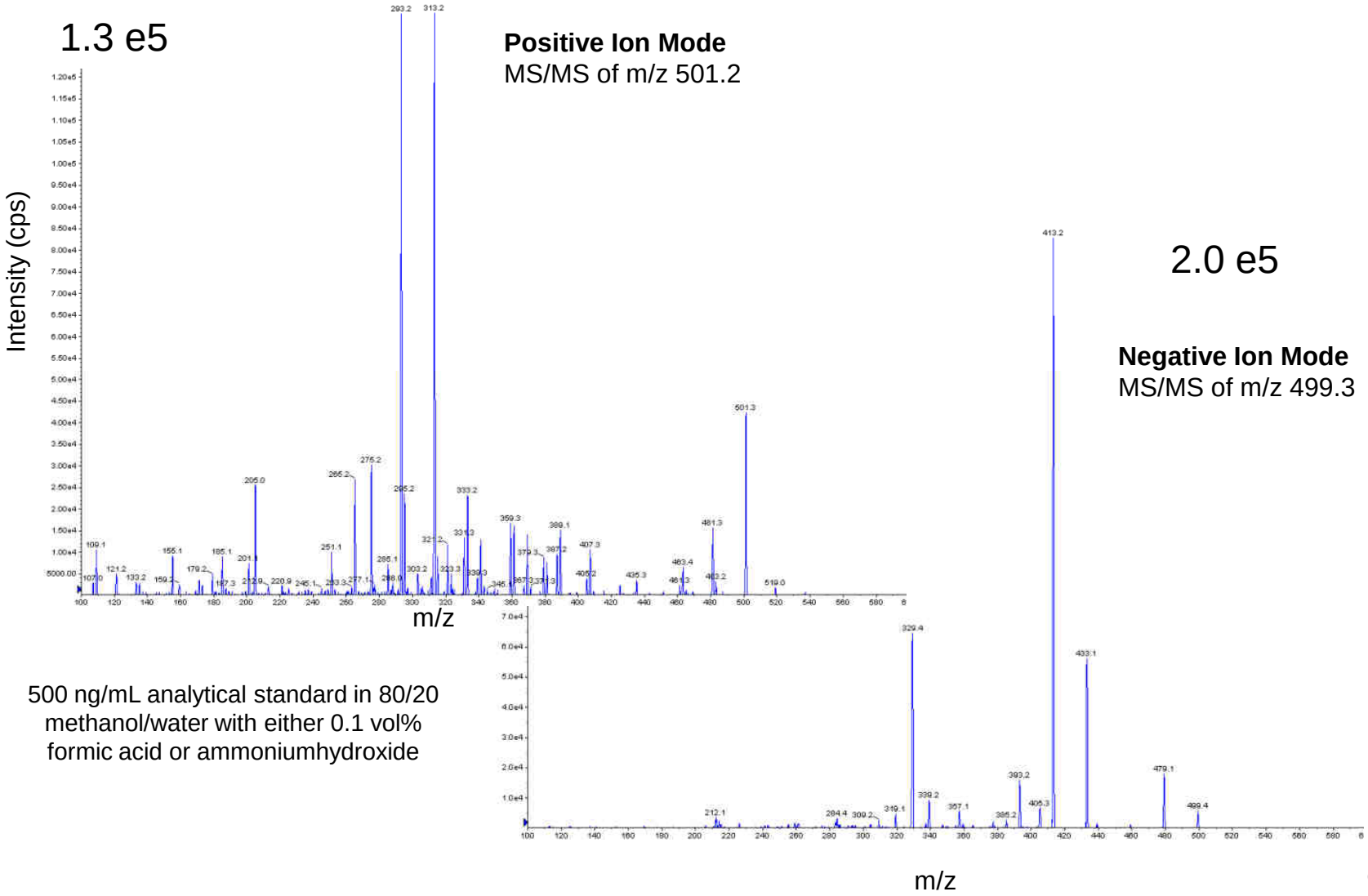
– Chemical structure and experimental approach –

- Fluticasone is a synthetic corticosteroid, used in asthma treatment and applied as topical drug
- Lung model used was an ex-vivo, mechanically perfusated and vented guinea pig lung
- 3 mg Fluticasone in 5 mL air was sprayed into the trachea of the lung model, simulating an inhaler set-up
- An AB/Sciex 5500 Qtrap MS with a Triversa NanoMate was used for LESA experiments
- LESA experiments were done with 80/20 methanol/water using 0.1 vol% formic acid (positive ion mode) or 0.1 vol% NH_4OH (negative ion mode) as the modifier.
- MS data shown is the average cps signal intensity over a 2 min spray time



Example 1: Distribution of fluticasone in lung tissue

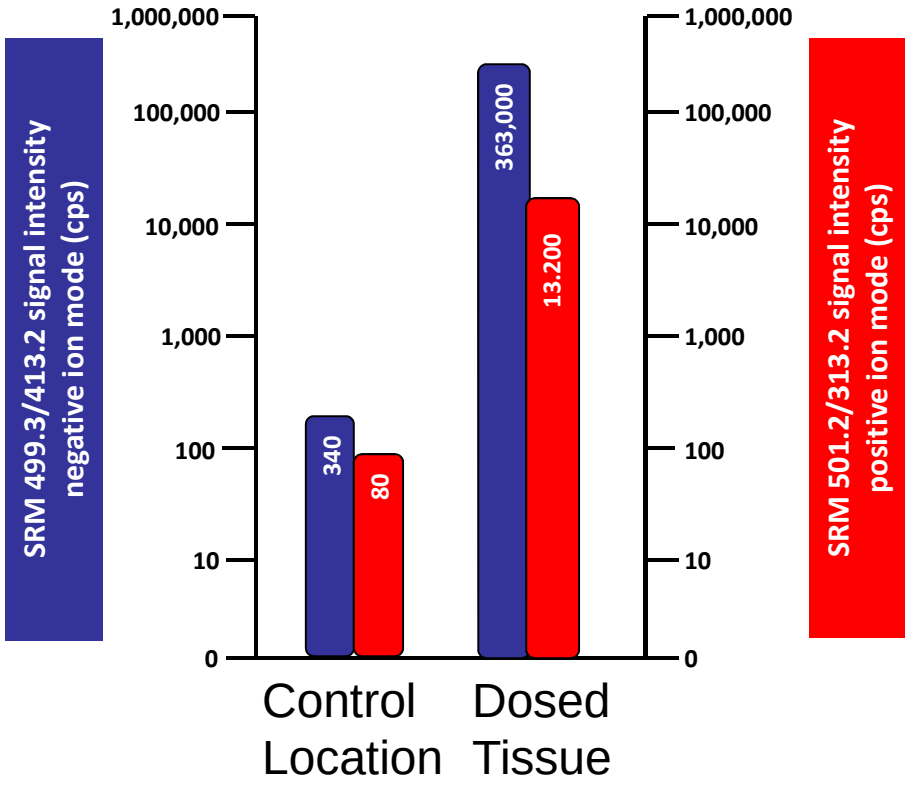
– Importance of ionization mode –



500 ng/mL analytical standard in 80/20 methanol/water with either 0.1 vol% formic acid or ammoniumhydroxide

Example 1: Distribution of fluticasone in lung tissue

– Importance of ionization mode –



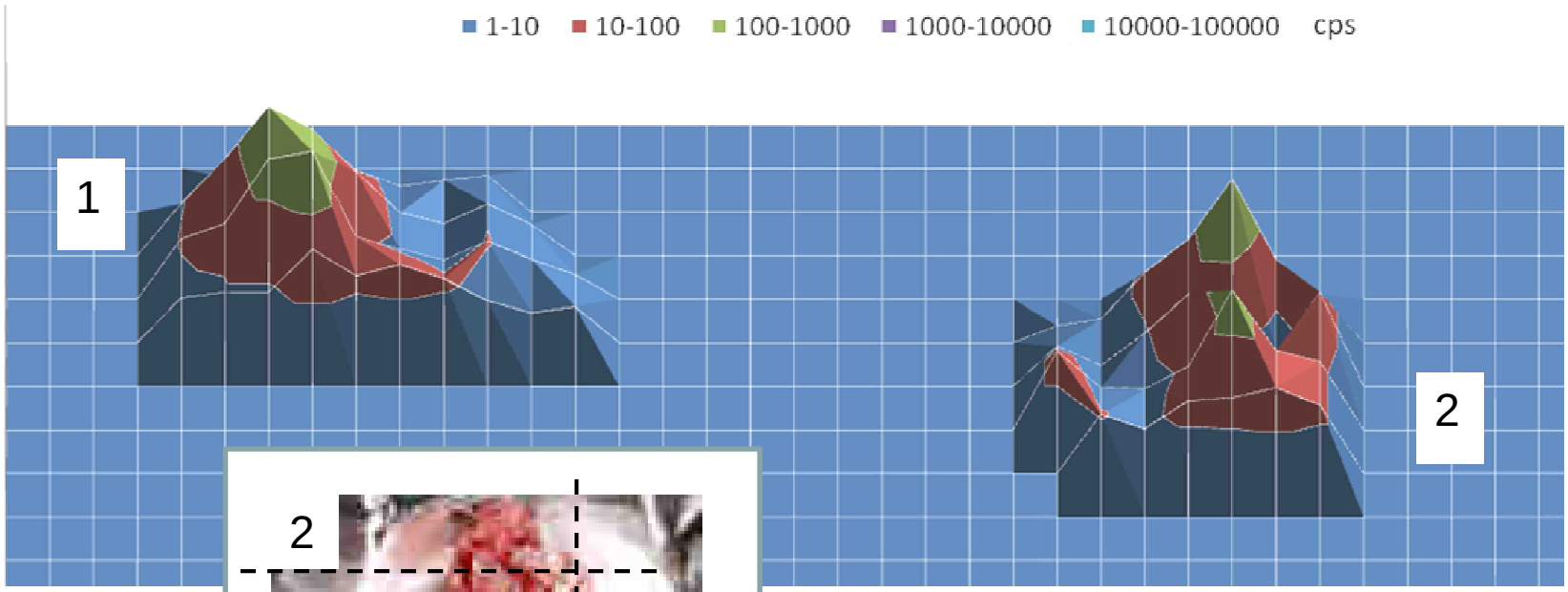
Negative ion mode SRM improvement, approx.

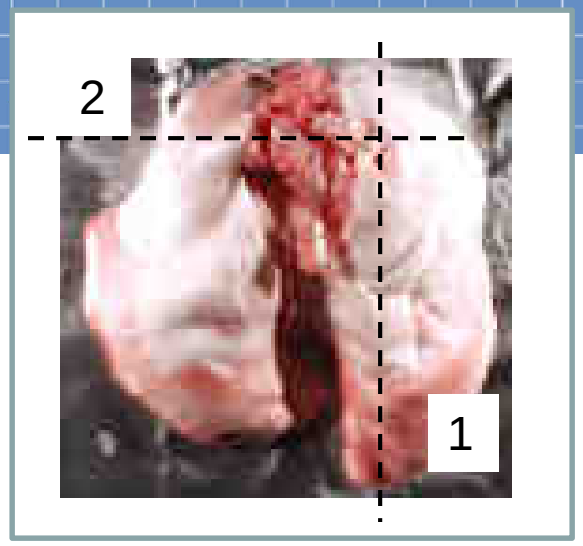
Signal: 27 fold

S/N: 6 fold

Example 1: Distribution of fluticasone in lung tissue

–Fluticasone is mainly distributed in the bronchial region of the lung –





Using Excell topographicmap function to represent LESA intensity data of Fluticasone SRM data collected, please note that Excell does interpolate data (colors) between the cross sections

Example 2
Diclofenac and it’s metabolites

…in cooperation with …

Stefan Linehan and Dennis Heller (XenoBiotic Laboratories)
Keeley Murphy (Thermo Fisher Scientific)
Patrick Rudewicz (Elan Pharmaceuticals)
and Jack Henion (Advion)

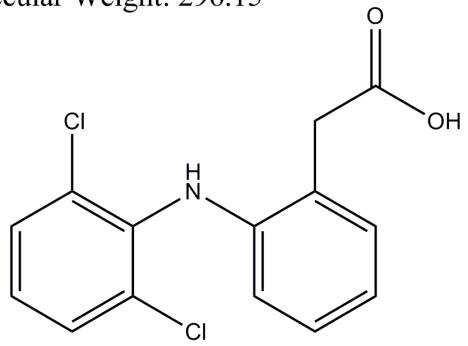
Example 2: Diclofenac and it's metabolites

– Chemical Structures and Experimental Approach–

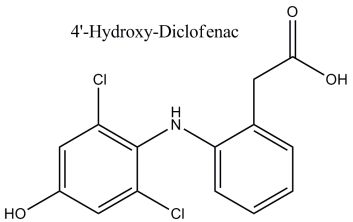
- Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) with a known and extensive metabolism
- Mouse was dosed with 15 mg/kg per oral, sacrificed after 15 min, blocked and sectioned
- An Exactive Benchtop Orbitrap MS with a Triversa NanoMate was used for LESA experiments
- LESA experiments were done with 80/20 methanol/water using 0.1 vol% NH_4OH as the modifier.
- MS data shown is the average of 1 min spray time (ca. 50 scans summed in max resolution and sensitivity)

Diclofenac

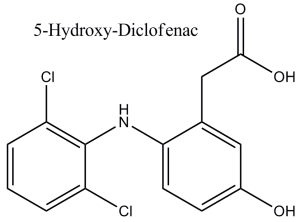
Chemical Formula: $C_{14}H_{11}Cl_2NO_2$
Exact Mass: 295.02
Molecular Weight: 296.15

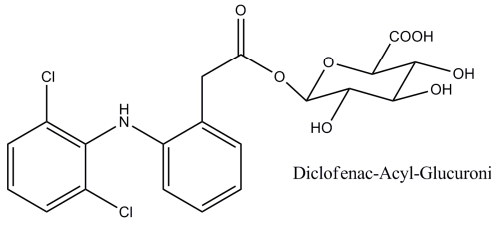


4'-Hydroxy-Diclofenac



5-Hydroxy-Diclofenac

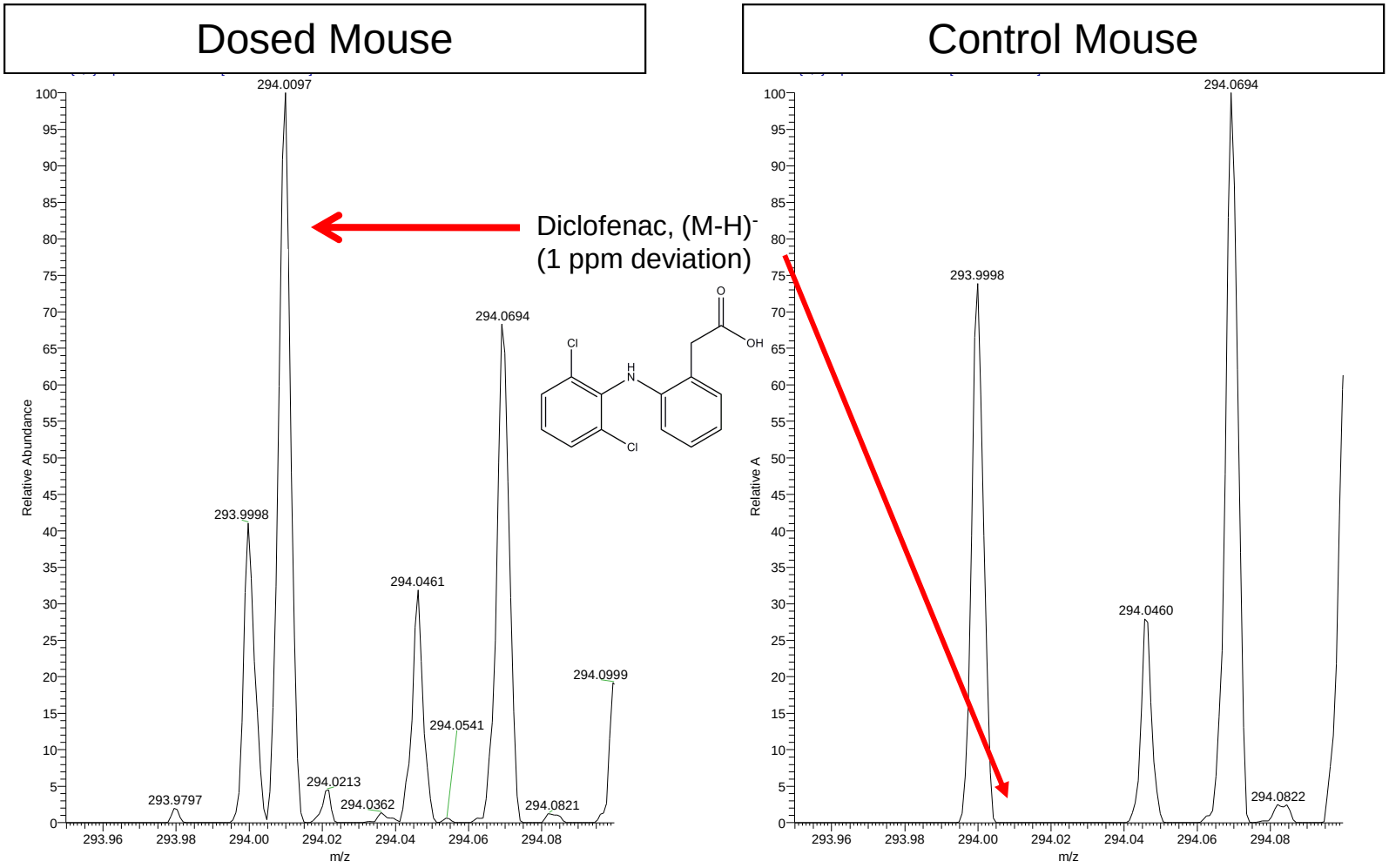




Diclofenac-Acyl-Glucuronide

Example 2: Diclofenac and it's metabolites

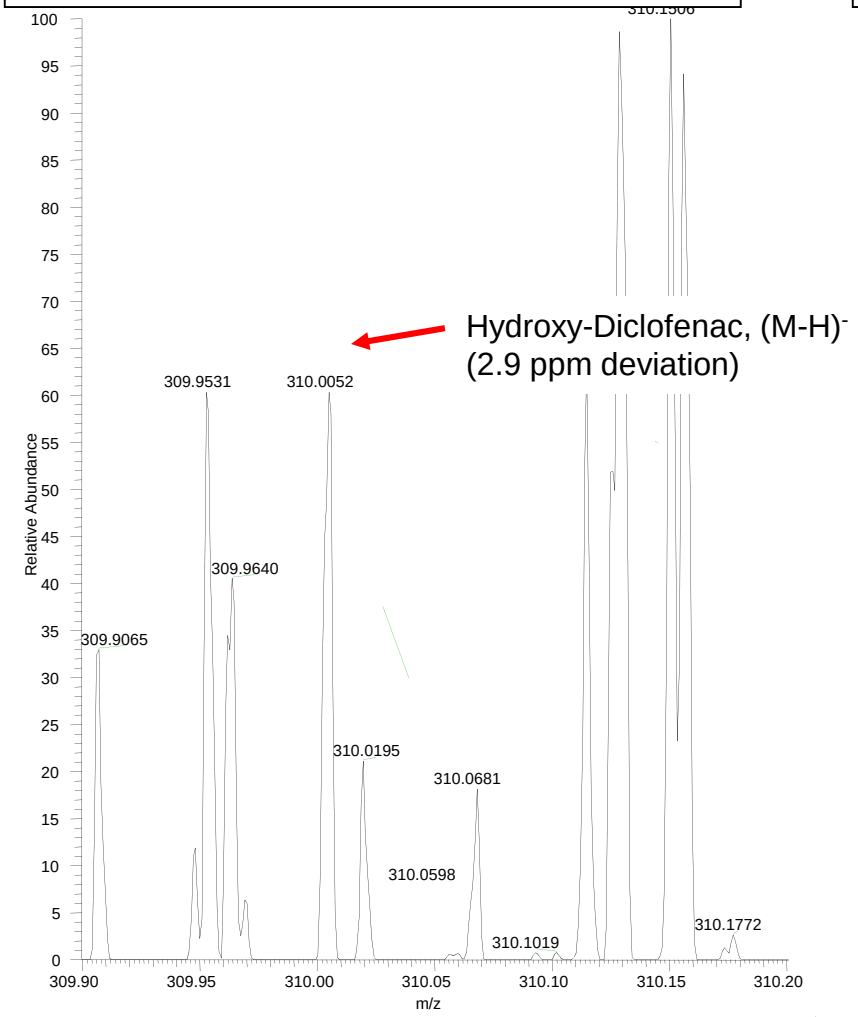
– Diclofenac parent drug in mouse brain –



Example 2: Diclofenac and it's metabolites

– Hydroxy-Diclofenac metabolite in mouse kidney –

Dosed Mouse



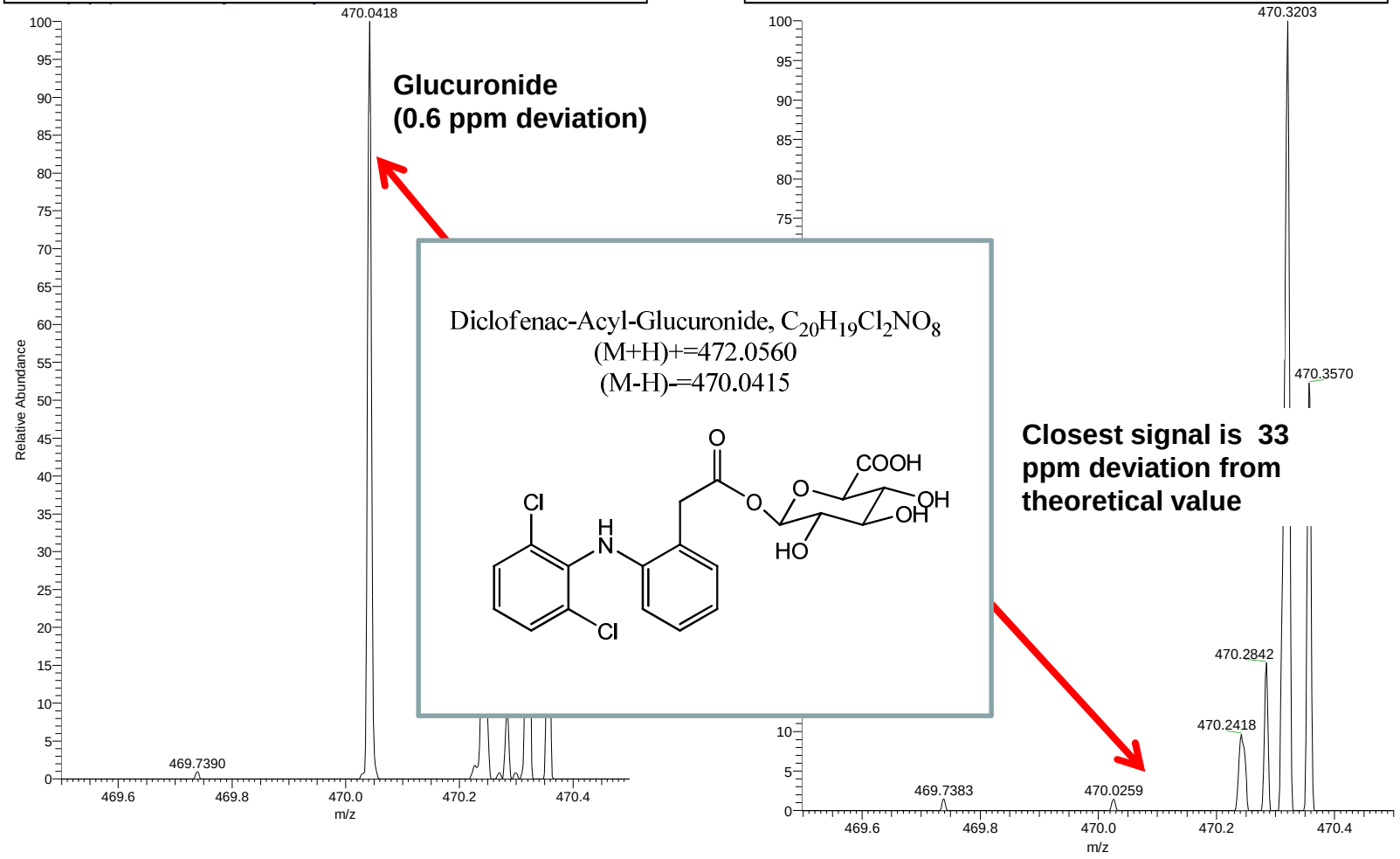
Control Mouse

Example 2: Diclofenac and it's metabolites

– Phase II glucuronide metabolite in liver –

Dosed Mouse

Control Mouse



Key points of the LESA technology

- shows a spatial resolution on target of about 1 mm
- uses only 1-2 µL of extraction solvent (static micro extraction, optimal area to volume ratio to increase analyte concentration in the nESI spray)
- employs nESI-chip technology to generate nESI for 2 - 8 min (ca. 300 nL/min flow through the nESI chip)
- extraction liquid can be composed of a wide variety of different solvents and modifiers (e.g. 80/20 Methanol/water 0.1 vol% formic acid)
- can easily be applied to compounds ionizing better in negative ion mode MS
- is able to detect parent drugs as well as phase I and II metabolites
- does not use HPLC separation (simple and fast, however might require MS based analyte separation such as ion mobility or MS/MS or high mass resolution capability)

Example 3

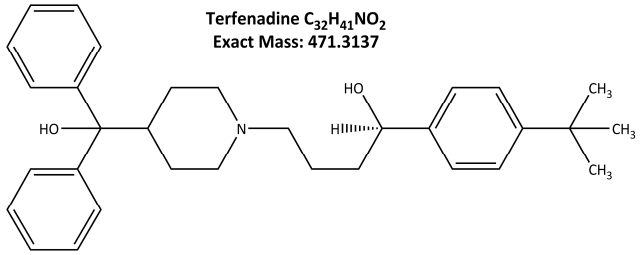
Distribution of Terfenadine and its metabolite Fexofenadine in whole body sections of mouse

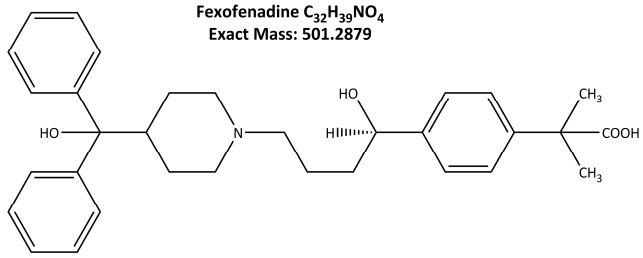
Eikel D, Vavrek M, Smith S, Bason C, Yeh S, Korfmacher WA and Henion JD:
Liquid Extraction Surface Analysis Mass Spectrometry (LESA-MS) as a Novel Profiling Tool for Drug Distribution and Metabolism Analysis: The Terfenadine Example.
Rapid Communication in Mass Spectrometry 2011 Dec 15;25(23):3587-96

Distribution of terfenadine and its metabolite fexofenadine in whole body sections of mouse

– Chemical Structures and Experimental Approach–

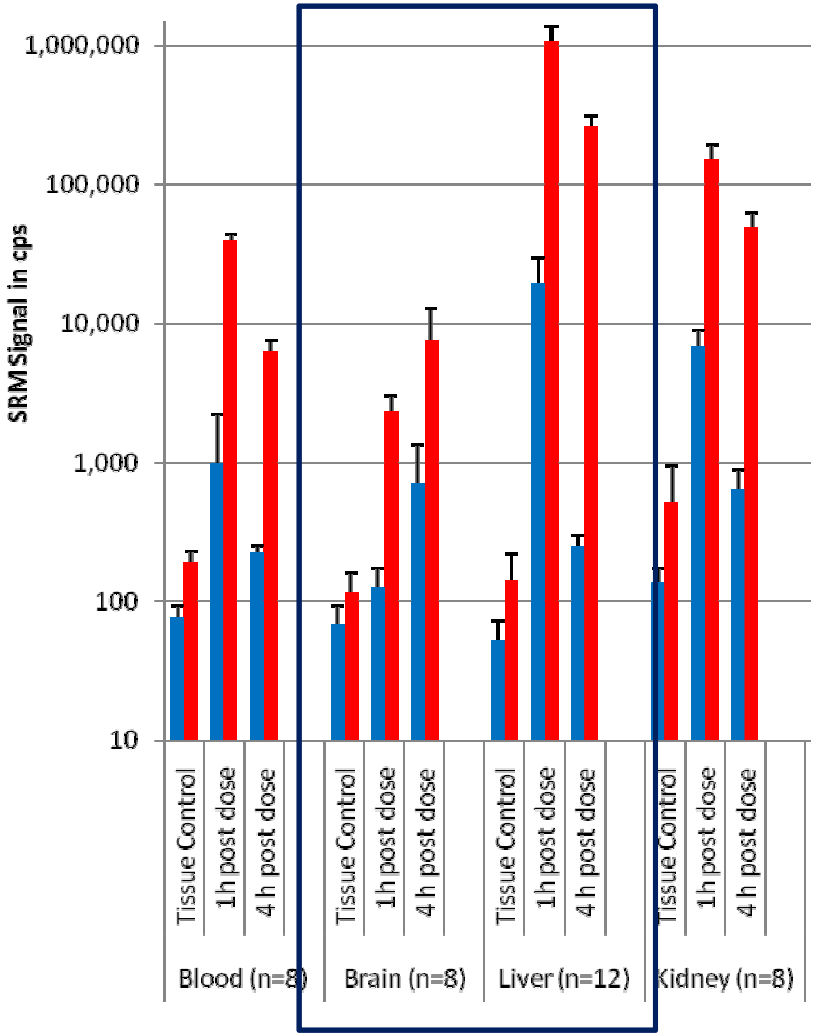
- Terfenadine and its metabolite Fexofenadine are antihistamines used for allergy treatment (Terfenadine is discontinued)
- Mouse was dosed with 50 mg/kg per oral, sacrificed in CO2 after 2 or 4 h, blocked and sectioned at 20 µm thickness
- An AB/Sciex 5500 Qtrap MS with a Triversa NanoMate was used for LESA experiments
- LESA experiments were done with 80/20 methanol/water using 0.1 vol% formic acid as the modifier.
- MS data shown is the average signal intensity of the SRM chanel in cps over 2 min (Terfenadine SRM 472.3/436.4; Fexofenadine SRM 502.3/171.1)





Distribution of terfenadine and its metabolite fexofenadine in whole body sections of mouse

– Distribution in whole body sections of mouse after 50 mg/kg PO dose –



Blue – Terfenadine (SRM 472.3/436.4)

Red – Fexofenadine (SRM 502.3/171.1)

Distribution of terfenadine and its metabolite fexofenadine in whole body sections of mouse

– Comparing LESA to other 'gold standards' –

Organ/Tissue	LESA-MS (QQQ) 50 mg/kg P.O. mouse 1h post dose		3H-Terfenadine study* 50 mg/kg P.O. mouse 1h post dose (³H-eq-ng/g)	14C-Terfenadine study* P.O rat [24] **Leeson et al. 1982**	
	Terfenadine	Fexofenadine		QWBA-6mg/kg 1h post dose	TLC-Rad- 10 mg/kg 2h post dose (14C-eq-ng/g)
Blood/ Heart	+	++	<LOQ	none	340 / 1500
Brain	0	+	<LOQ	none	
Liver	++	++++	113	weak	19200
Kidney	+	+++	59	none	4500
Spleen	+	+++	<LOQ	none	3800
Skeletal muscle	+	+	<LOQ	none	
Stomach	+++	++	9363	strong	
Small Intestine	++	+++	1011	strong	
Large Intestine	0	++	1744	none	

Considering ESI matrix effects, LESA shows a surprisingly good correlation to QWBA comparison data

Distribution of terfenadine and its metabolite fexofenadine in whole body sections of mouse

– Comparing to other ‘gold standards’ –

Organ/Tissue	LESA-MS (QQQ) 50 mg/kg P.O. mouse 1h post dose		LC-MS/MS (QQQ) 50 mg/kg P.O. mouse 1h post dose (nmol/g)	
	Terfenadine	Fexofenadine	Terfenadine	Fexofenadine
Blood/ Heart	+	++	0.6	0.5
Brain	0	+	5.2	0.5
Liver	++	++++	1174.0	49.0
Kidney	+	+++	17.5	6.0
Spleen	+	+++	13.3	0.4
Skeletal muscle	+	+	44.4	0.8

LESA shows nmol/g detection limit
(of course matrix and compound dependant)

Distribution of terfenadine and its metabolite fexofenadine in whole body sections of mouse

– Comparing to other ‘gold standards’ –

Organ/Tissue	LESA-MS (QQQ) 50 mg/kg P.O. mouse 1h post dose		MALDI-MSI 50 mg/kg P.O. mouse 1h post dose [25] (signal intensity) Chen et al. 2008	
	Terfenadine	Fexofenadine	Terfenadine	Fexofenadine
Blood/ Heart	+	++	none	none
Brain	0	+		
Liver	++	++++	none	medium
Kidney	+	+++	none	none
Spleen	+	+++		
Skeletal muscle	+	+	none	none
Stomach	+++	++	medium	none
Small Intestine	++	+++	strong	strong
Large Intestine	0	++	none	none

LESA shows favorable sensitivity compared to MALDI imaging

Some more key points of the LESA technology

- Sample analysis can be quick (about 1 min) or can be extended for more complex MS analysis (signal averaging, data dependant MS experiments ect.)
- does not require additional sample preparation
- nmol/g detection limit of LESA (matrix and compound dependant)
- favorable sensitivity compared to MALDI imaging
- Although comparing different tissues with each other should be difficult because of matrix effects in ESI, LESA data is a surprisingly good correlation to other gold standards such as QWBA and LC-MS/MS

Summary/Conclusions

- LESA allows a quick overview of drug distribution within a whole body animal section as well as a single organ profiling

- It is quite sensitive, it suffers less from matrix suppression than expected and allows distribution analysis across tissues

- LESA-MS provides a novel and alternative approach to investigate drug distribution and metabolism in early drug development requiring little sample preparation and no radiolabeled material

Acknowledgements

- Thanks to all the cooperation partners mentioned throughout the presentation
- Especially AB/Sciex and Thermo Fisher Scientific for the generous loan of a 5500 Qtrap and an Exactive Benchtop Orbitrap mass spectrometer respectively.

- Thank you very much for your time and interest in the LESA technology !

- A 20 min Q+A online session will follow after this presentation, please post your questions in the communication section on the right side of your webinar view window

Advion