

Liquid Extraction Surface Analysis Mass Spectrometry (LESA MS)

-
Drug Distribution and Metabolism of Diclofenac
in the Mouse

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(4) Elan Pharmaceuticals Inc., CA, USA

Overview

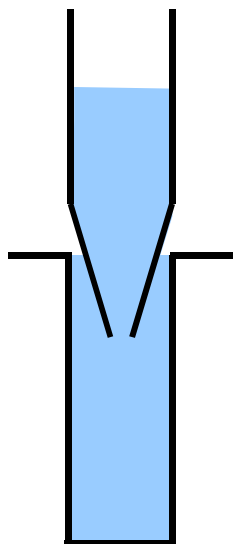
- Introduction to liquid extraction surface analysis mass spectrometry (LESA-MS)
- Example analysis of Diclofenac from thin whole body mouse sections
- Diclofenac distribution analysis and metabolite identification
- Summary and Conclusions

Introduction to LESA

– Schematic Workflow –

Step 1 – Solvent Delivery

Disposable tip picks up extraction solvent at reservoir



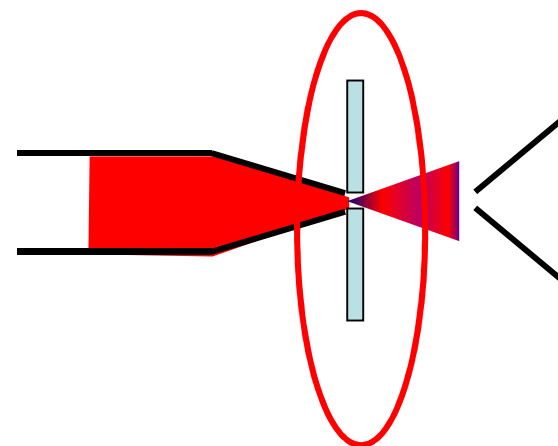
Step 2 – Analyte Extraction

Robot places extraction solvent on target and initiates dispense/dispense cycles for analyte extraction



Step 3 – Analyte Ionization

Robot aspirates extracted analytes from target and initiates electro spray at a 400 nozzle nano ESI chip

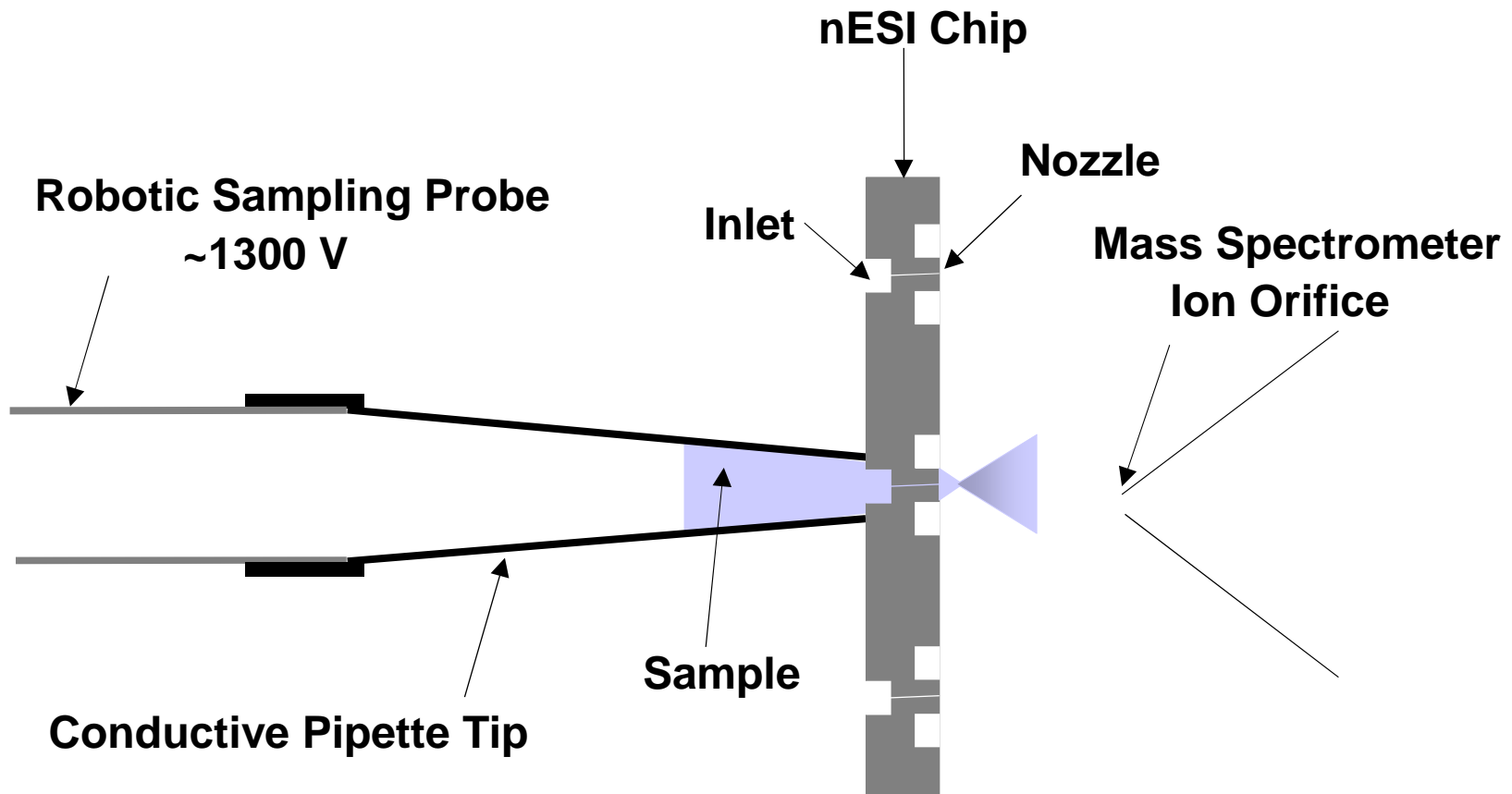


Chip-based
nano ESI

Kertesz V. and Van Berkel GJ: Fully automated liquid extraction-based surface sampling and ionization using a chip-based robotic nano electro spray platform. *Journal of Mass Spectrometry* 2010 45(3) p252-260

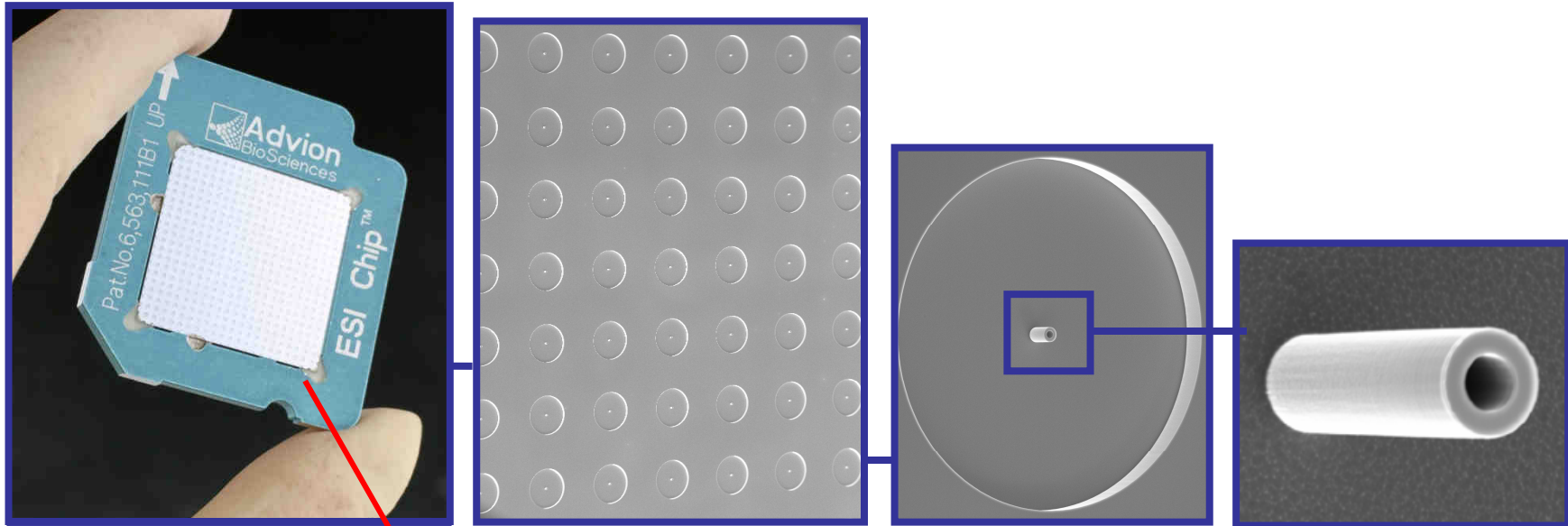
Introduction to LESA

– Schematic of Tip-to-nESI-Chip –



Micro Fabricated nano ESI chip

– 400 nozzles/samples per Chip –



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

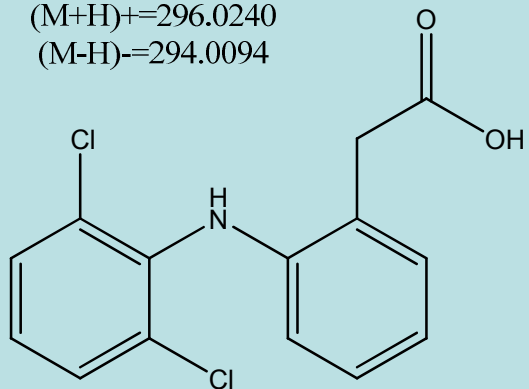


**Universal
sample support
holder platform**

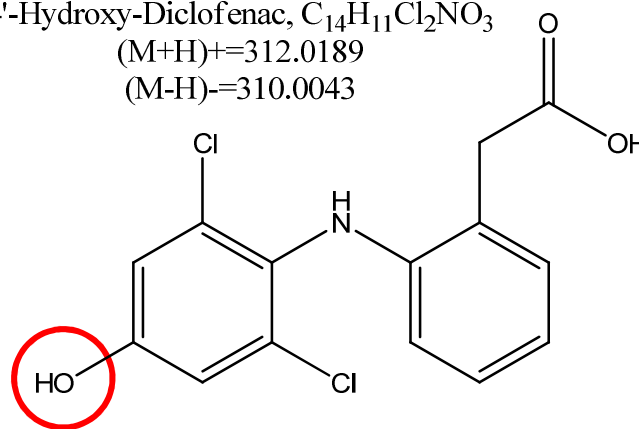
Chemical Structures

– Diclofenac and selected Metabolites –

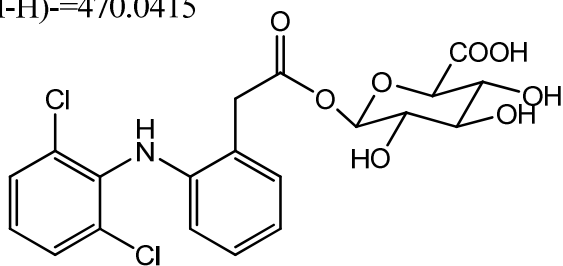
Diclofenac, $C_{14}H_{11}Cl_2NO_2$
(M+H) $^+$ =296.0240
(M-H) $^-$ =294.0094



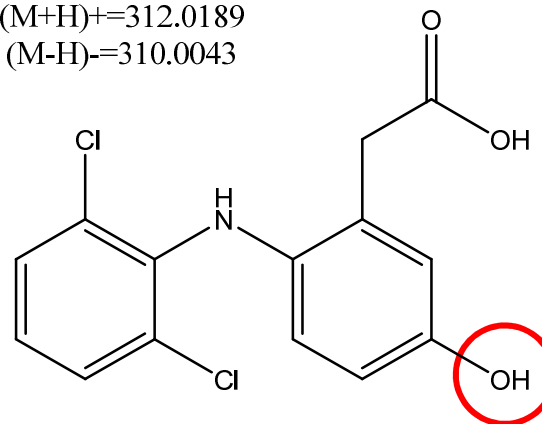
4'-Hydroxy-Diclofenac, $C_{14}H_{11}Cl_2NO_3$
(M+H) $^+$ =312.0189
(M-H) $^-$ =310.0043



Diclofenac-Acyl-Glucuronide, $C_{20}H_{19}Cl_2NO_8$
(M+H) $^+$ =472.0560
(M-H) $^-$ =470.0415



5-Hydroxy-Diclofenac, $C_{14}H_{11}Cl_2NO_3$
(M+H) $^+$ =312.0189
(M-H) $^-$ =310.0043



Experimental

- **Male balb-C mouse (18-26 g)**
 - P.O. dosed with 10 mg/kg Diclofenac (or vehicle only for control mouse),
 - Sacrificed after 15 min in CO₂, blocked and sectioned in cryomicrotome
- **LESA parameters:**
 - Extraction solvent 80/20 Methanol/Water
 - 0.1 vol % formic acid for positive ion mode
 - 0.1% Ammonium hydroxide for negative ion mode.
 - 1.5 µL extraction solvent/pick up
 - 1.1 µL dispense followed by aspiration of 1.3 µL from the tissue, repeat dis/asp 2 more times.
 - nESI generated using 0.9 psi and -1.25 kV or 0.3 psi and 1.35 kV respectively.
- **Thermo Exactive Benchtop Orbitrap**
 - calibrated daily and tuned for m/z 195.0877 and m/z 265.1479 in positive and negative ion mode respectively
 - operated at 100,000 nominal resolution with AGC set to high sensitivity (5e6), 250 ms fill time limit.
- **AB SCIEX 5500 QTRAP**
 - Tuned and calibrated per manufacturer's recommendations
 - Operated in Enhanced Product Ion (EPI) Mode using optimal collision energy for the respective compound as determined by analytical standard

LESA Points Software

Example for sequence list generation –

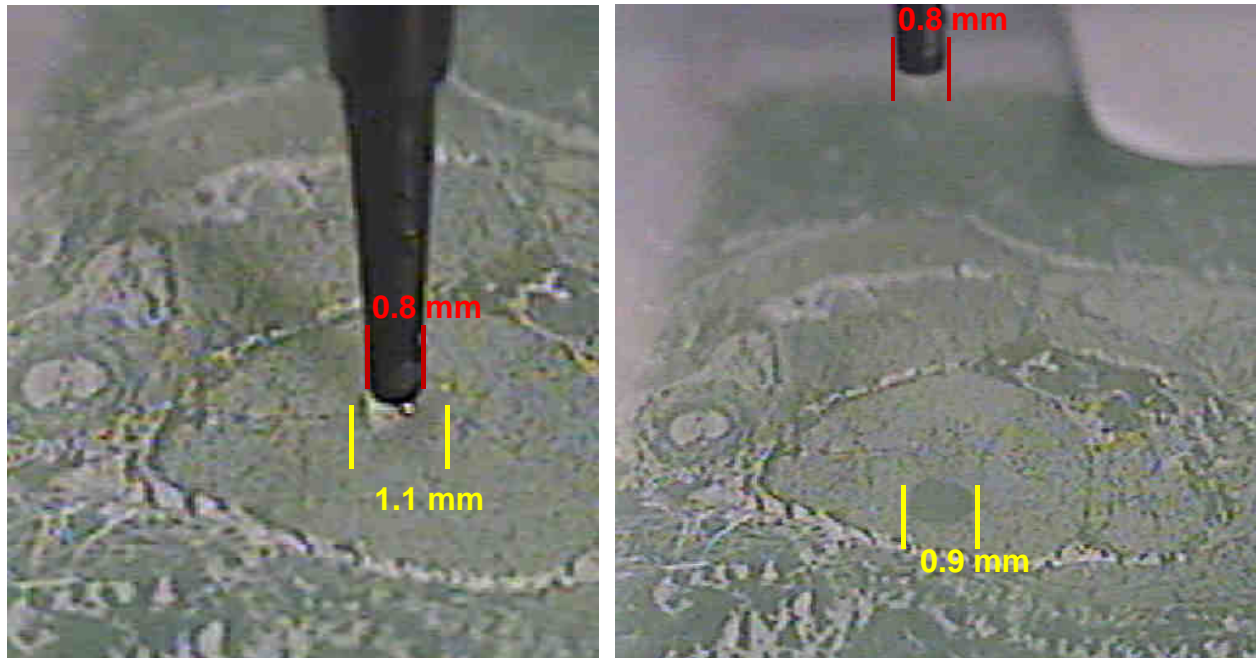
The screenshot displays the LESA Points software interface. The main window shows an anatomical image of a pig with several blue circular points marked on it. Four white boxes with black arrows point to these points, labeled: "Intestinal Content", "Stomach Content", "Brain", and "Liver".

On the right side, there is a "Sequence" panel containing a table with three columns: "Location", "Sample Desc", and "Method File". The table lists 17 entries corresponding to the points in the image.

Location	Sample Desc	Method File
L35:X-05:Y+06	Brain	C:\Program Files\Advion\ChipSoft\met...
L35:X+09:Y+09	Brain	C:\Program Files\Advion\ChipSoft\met...
M36:X-03:Y-08	Brain	C:\Program Files\Advion\ChipSoft\met...
M36:X+12:Y+01	Brain	C:\Program Files\Advion\ChipSoft\met...
Q24:X+04:Y+03	Liver	C:\Program Files\Advion\ChipSoft\met...
R24:X+02:Y-01	Liver	C:\Program Files\Advion\ChipSoft\met...
S24:X+0:Y-08	Liver	C:\Program Files\Advion\ChipSoft\met...
S24:X-05:Y+10	Liver	C:\Program Files\Advion\ChipSoft\met...
M23:X-02:Y-11	Stomach	C:\Program Files\Advion\ChipSoft\met...
M22:X+12:Y-01	Stomach	C:\Program Files\Advion\ChipSoft\met...
M22:X+0:Y+11	Stomach	C:\Program Files\Advion\ChipSoft\met...
N21:X+10:Y-08	Stomach	C:\Program Files\Advion\ChipSoft\met...
S20:X+08:Y-03	Intestine	C:\Program Files\Advion\ChipSoft\met...
Q20:X-02:Y+09	Intestine	C:\Program Files\Advion\ChipSoft\met...
S19:X+04:Y-07	Intestine	C:\Program Files\Advion\ChipSoft\met...
P17:X-08:Y+12	Intestine	C:\Program Files\Advion\ChipSoft\met...

LESA Spatial Resolution

Example of the Liquid Junction and Tissue Hydration



Spatial resolution depends on:

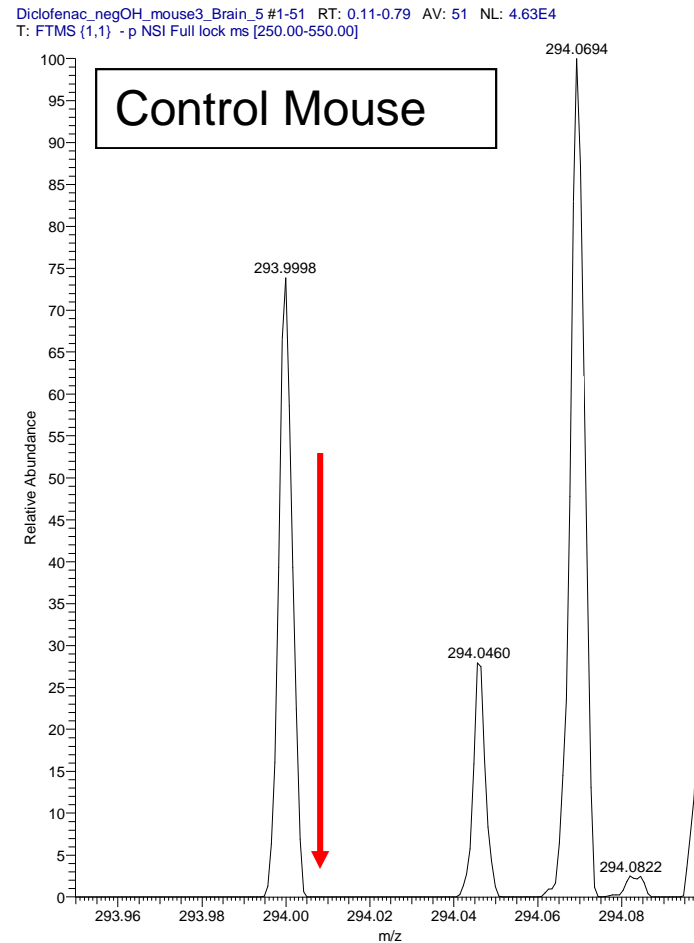
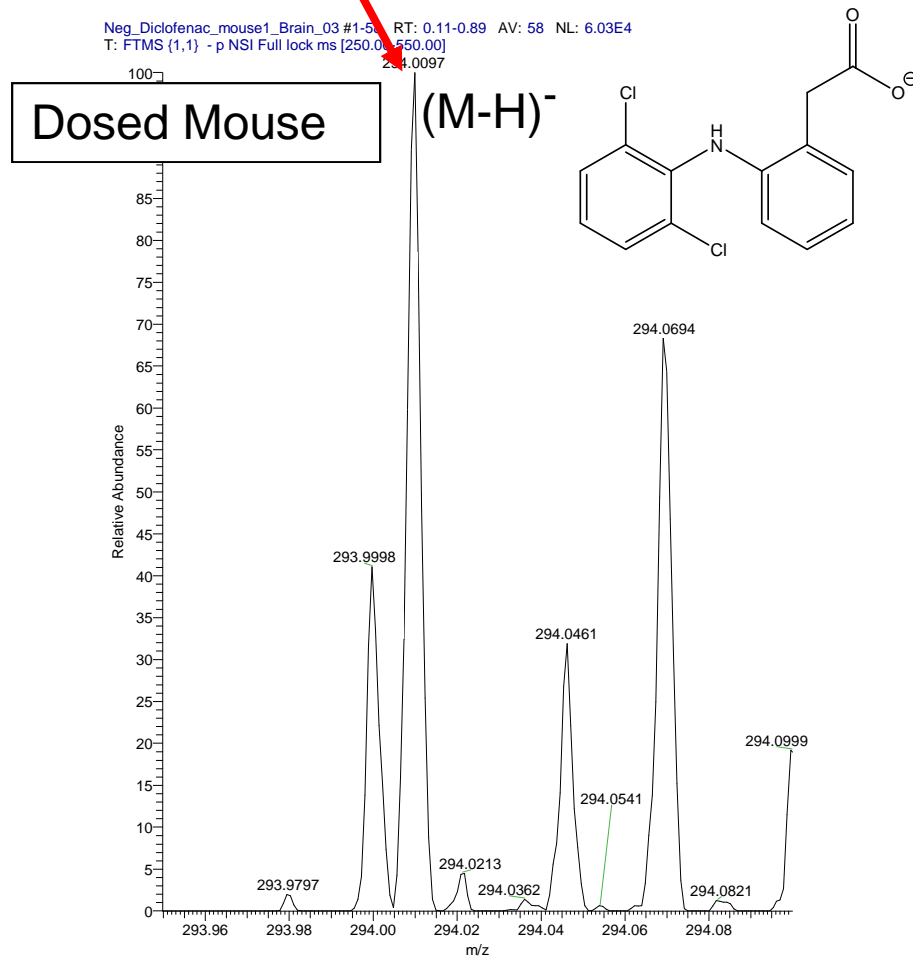
- Solvent Composition

- Volume dispensed on the surface

- Surface Tension (e.g. hydration state of the surface)

LESA MS Determination of Diclofenac: **Exactive** Brain: Negative Ions

Diclofenac
(1 ppm deviation)

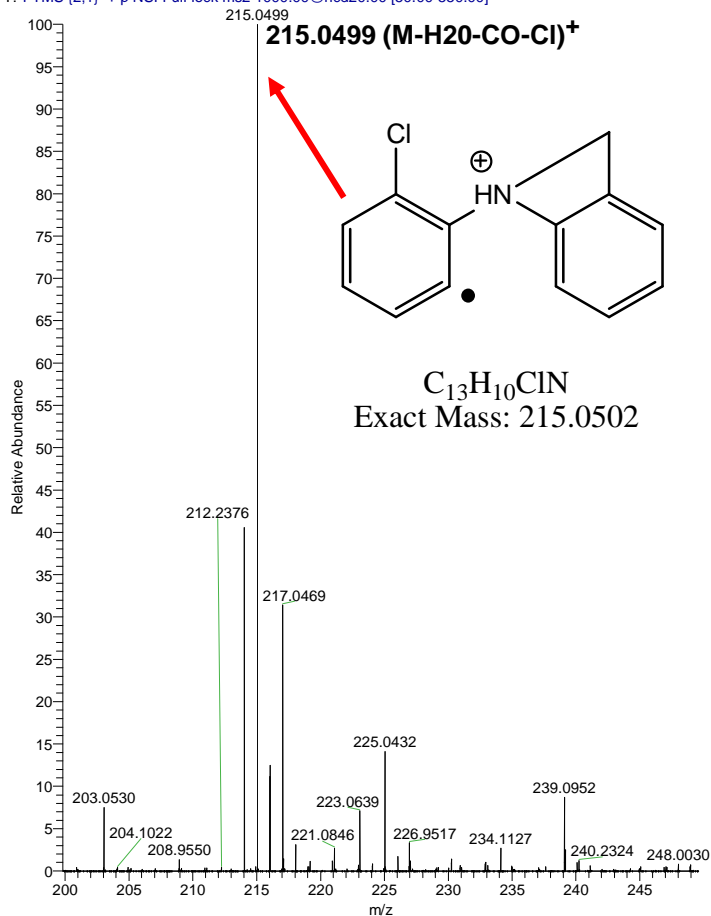


Parent Diclofenac readily observed in brain tissue with
Exactive at high resolution

LESA All-Ion MS/MS Diclofenac: **Exactive** Stomach: Positive Ions

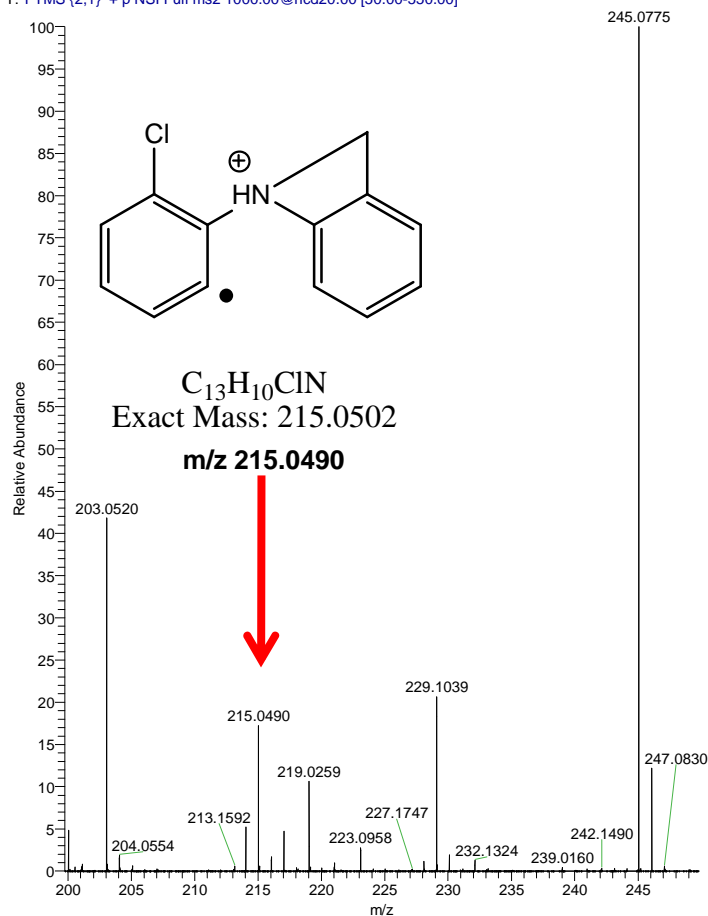
Diclofenac analytical standard

Diclofenac_pos_std #63-97 RT: 0.85-1.35 AV: 35 NL: 1.56E6
T: FTMS (2,1) + p NSI Full lock ms2 1000.00@hcd20.00 [50.00-550.00]



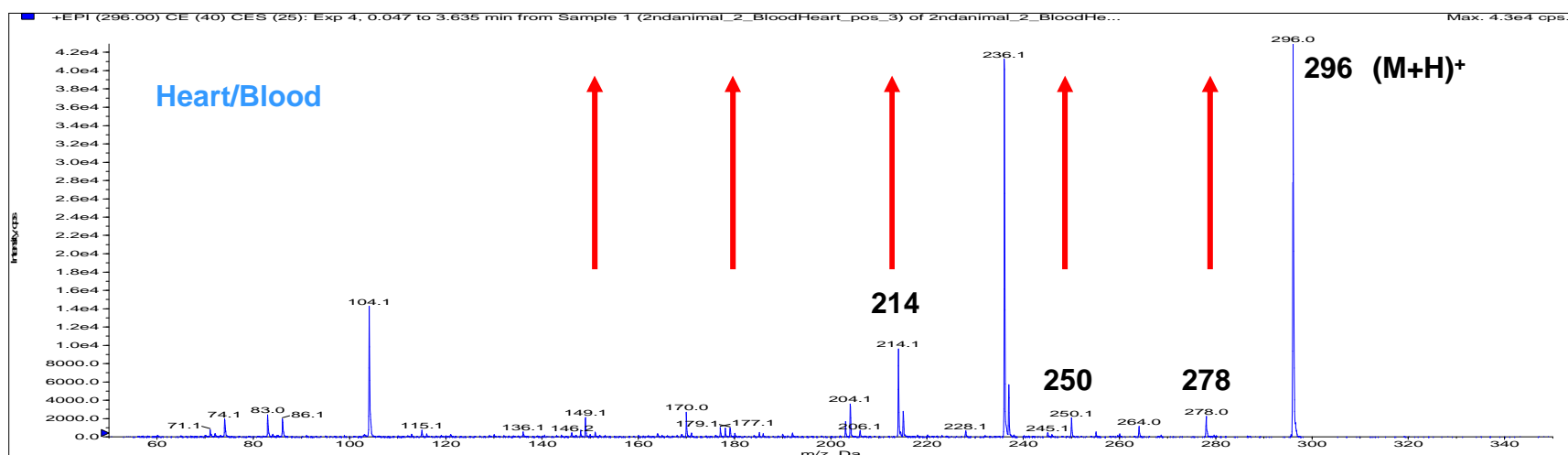
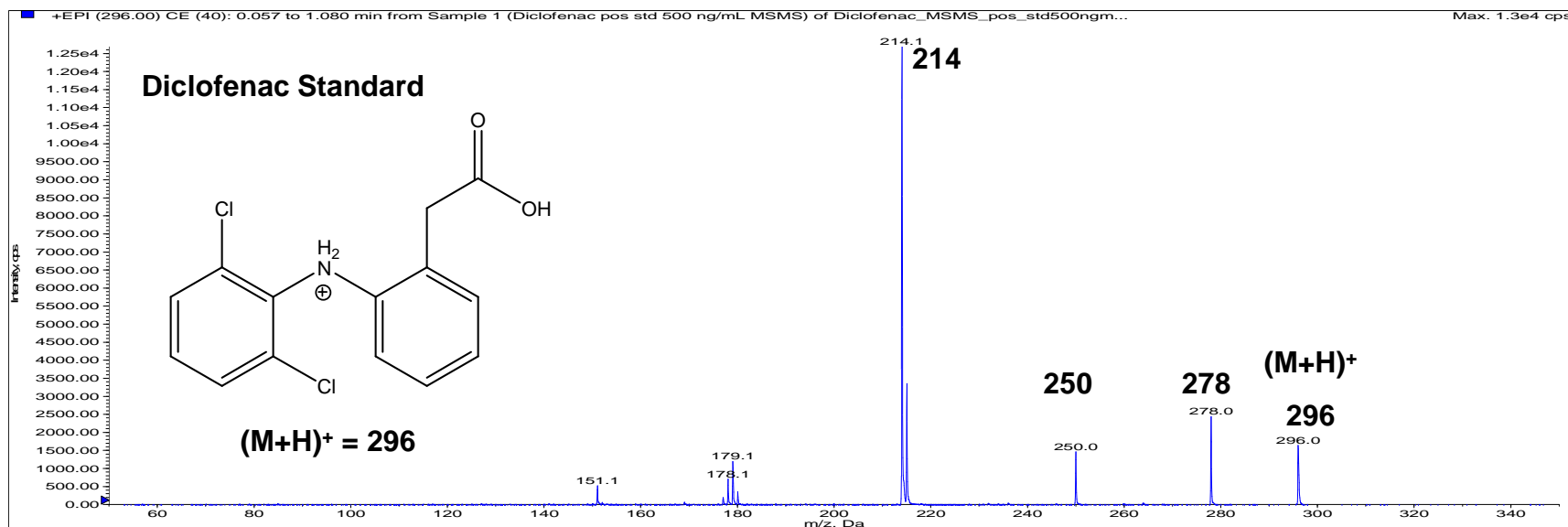
Stomach, 15 min post-dose

POS_Mouse1_Stomach_03 #62-103 RT: 0.95-1.53 AV: 42 NL: 1.22E7
T: FTMS (2,1) + p NSI Full ms2 1000.00@hcd20.00 [50.00-550.00]

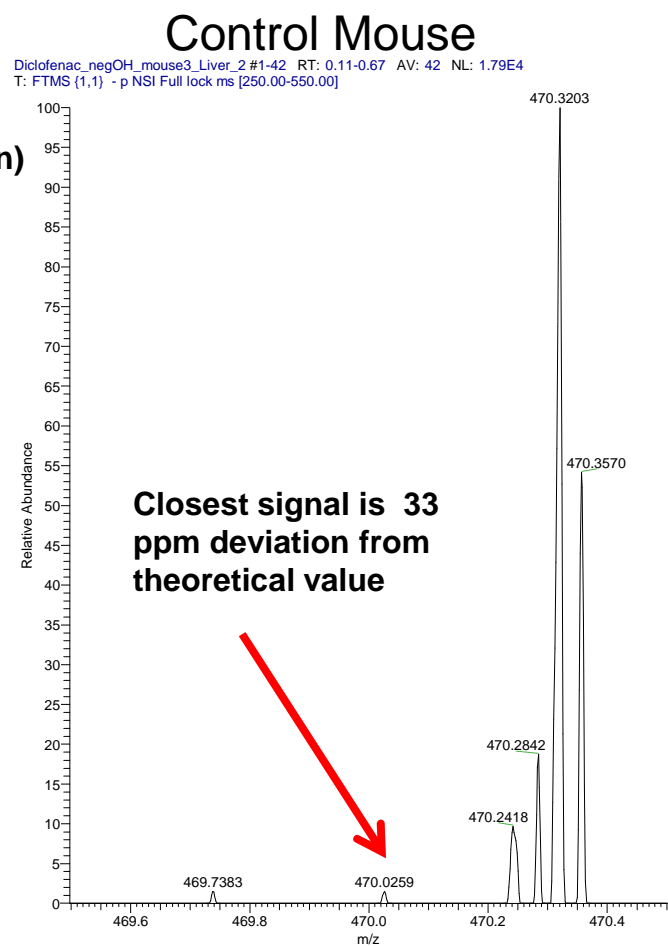
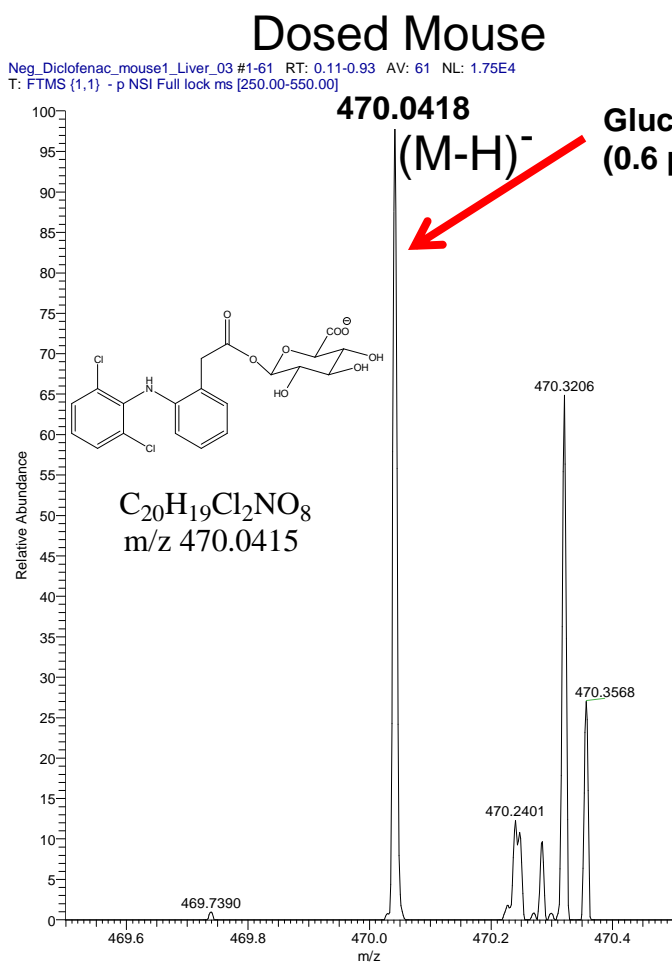


LESA MS/MS Confirmation of Diclofenac: 5500

Heart/Blood: Positive Ions

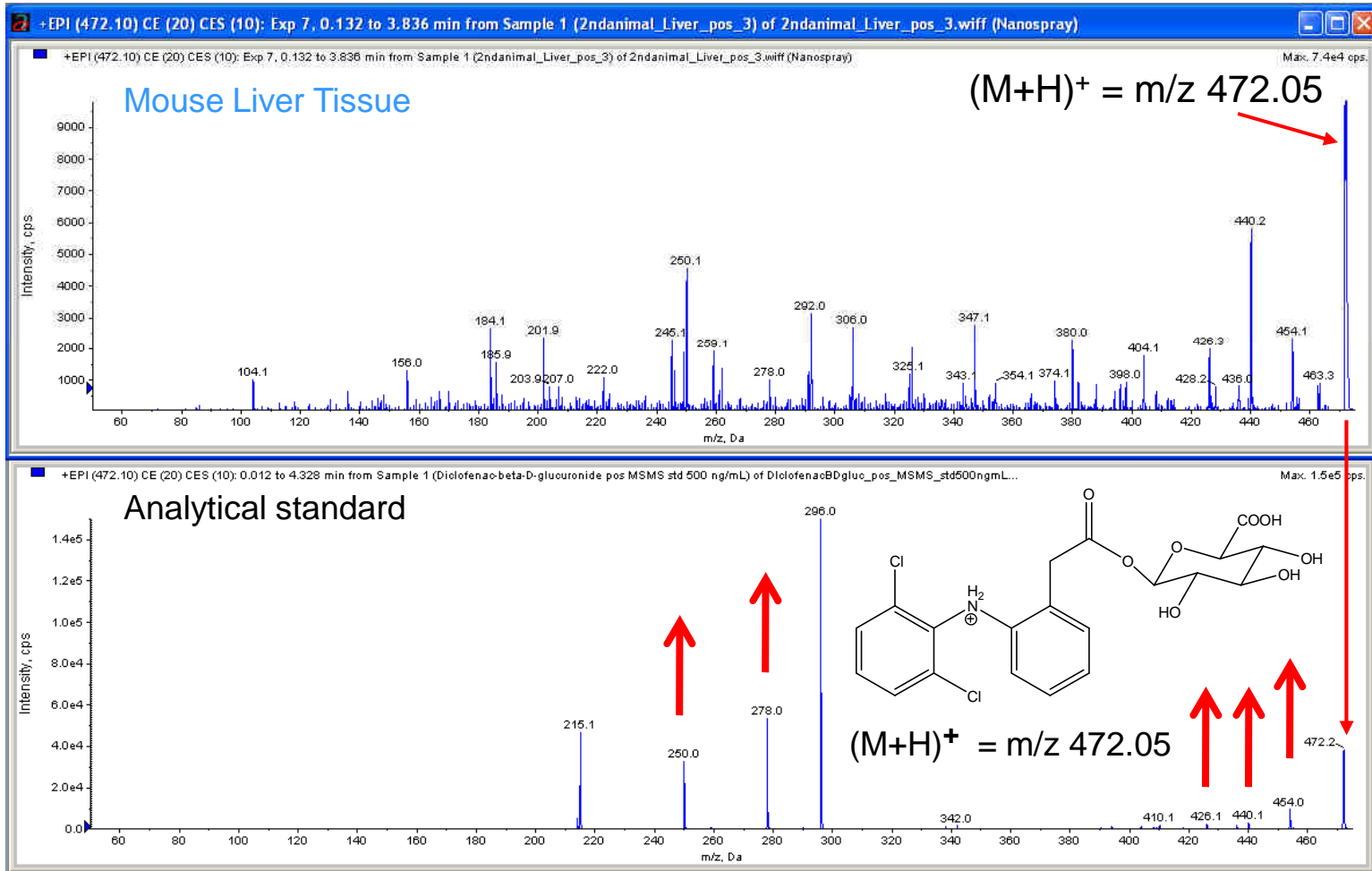


LESA MS Confirmation of Diclofenac-beta-D-glucuronide: **Exactive** Liver Tissue: Negative Ions



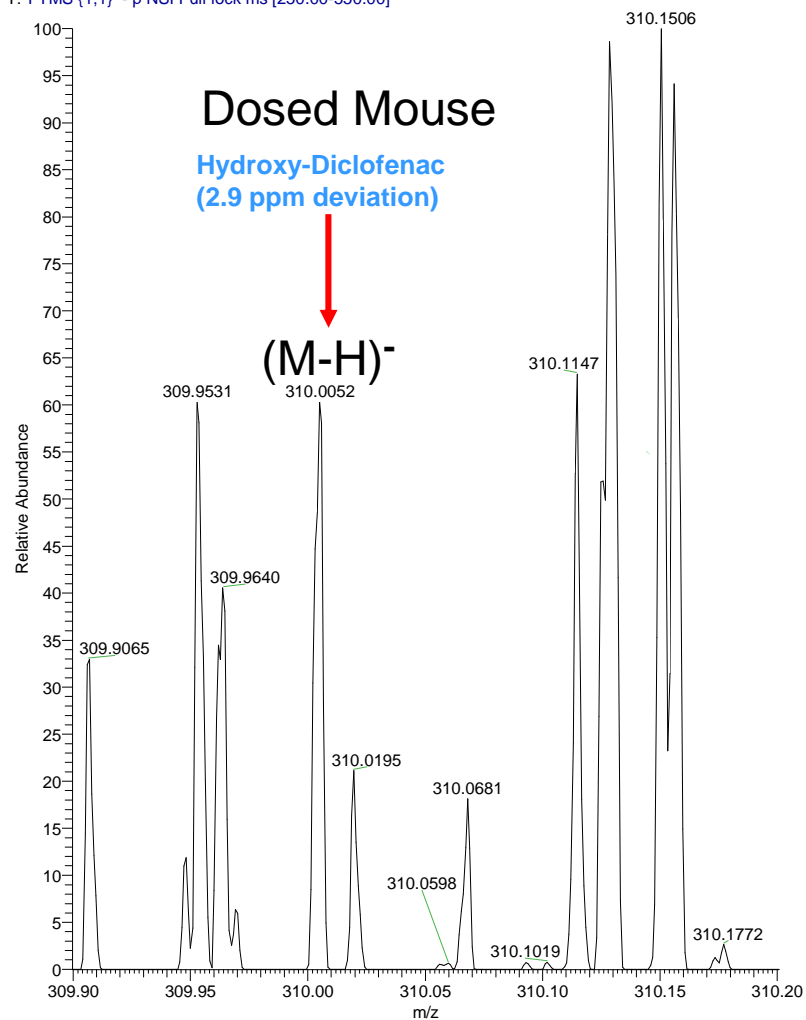
**Facile Detection of Glucuronide Metabolite in liver tissue
with high resolution MS**

LESA MS/MS Confirmation of Diclofenac-beta-D-glucuronide: 5500 Liver Tissue: Positive Ions

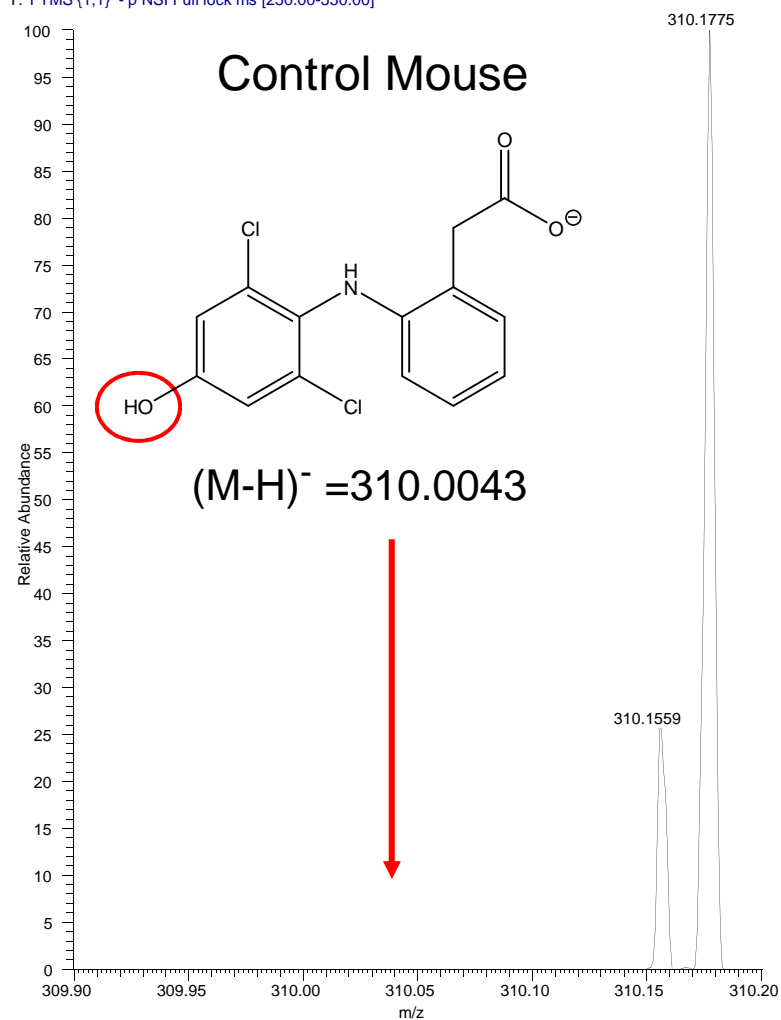


LESA MS Detection of Diclofenac Hydroxy Metabolites: **Exactive** Kidney Tissue: **Negative Ions**

Neg_Diclofenac_mouse1_Kidney_03 #1-53 RT: 0.11-0.83 AV: 53 NL: 1.57E4
T: FTMS (1,1) - p NSI Full lock ms [250.00-550.00]



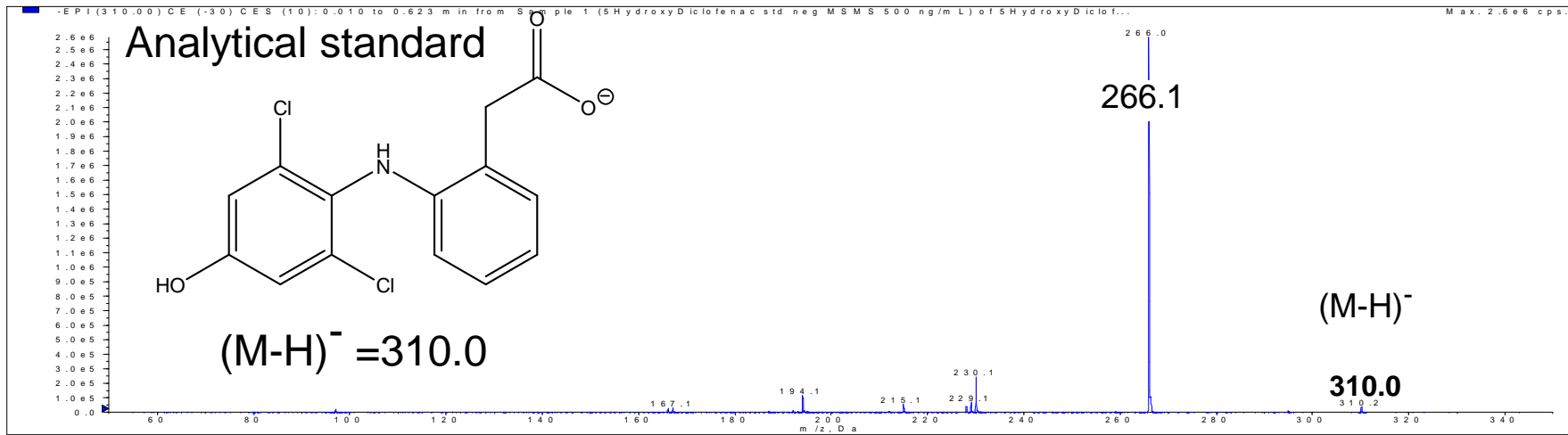
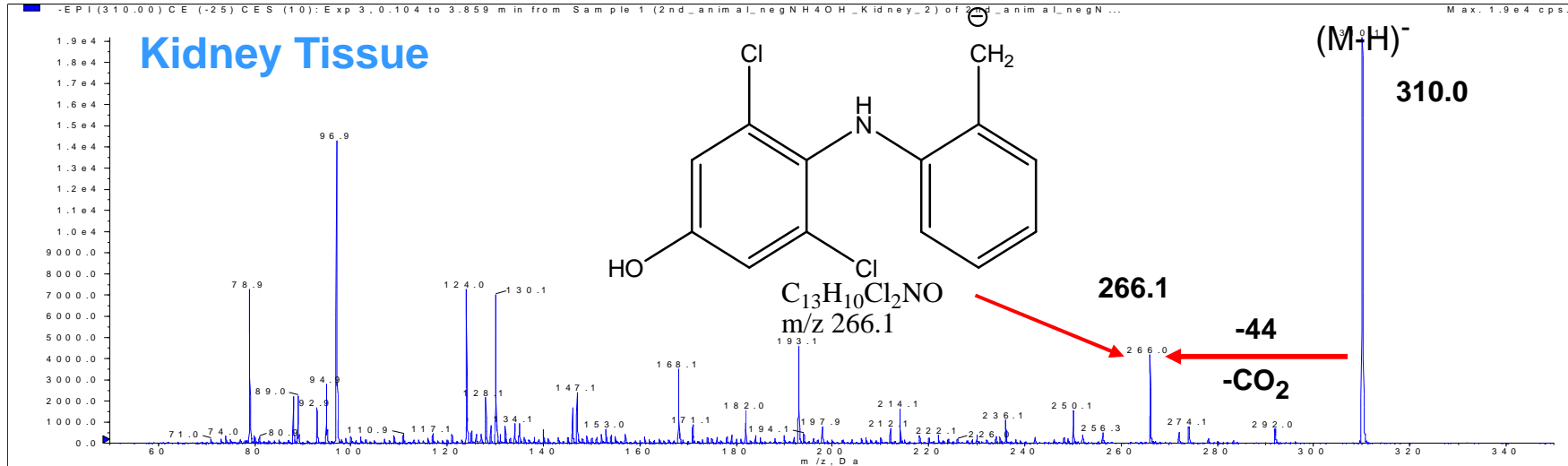
Diclofenac_negOH_mouse3_Kidney_6 #1-60 RT: 0.11-0.92 AV: 60 NL: 5.50E4
T: FTMS (1,1) - p NSI Full lock ms [250.00-550.00]



Facile Detection of Hydroxy Metabolite in kidney tissue with high resolution MS

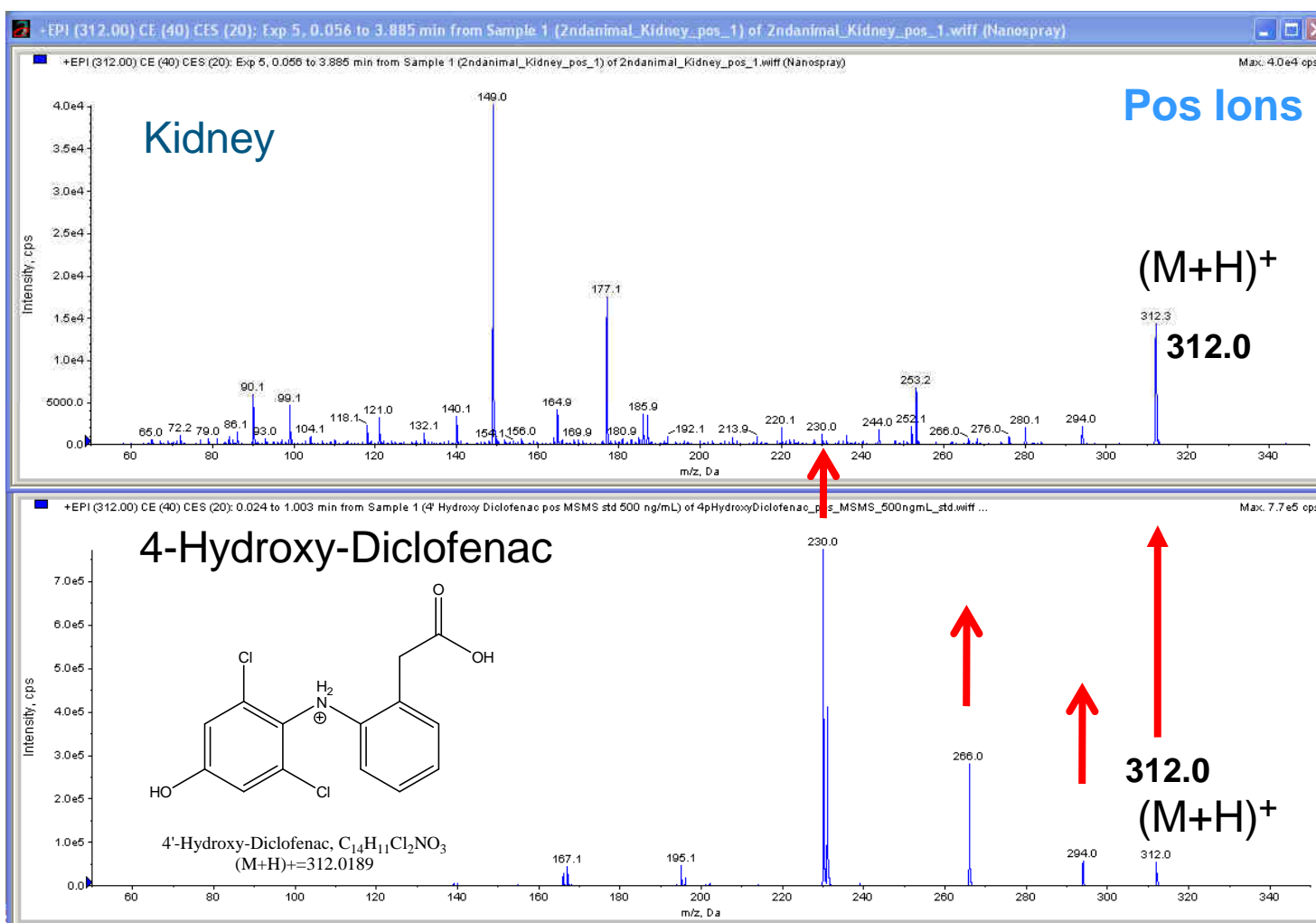
LESA MS/MS Detection of Diclofenac Hydroxy Metabolites : 5500

Kidney Tissue: Negative Ions



Negative ion MS/MS provides limited characterization information

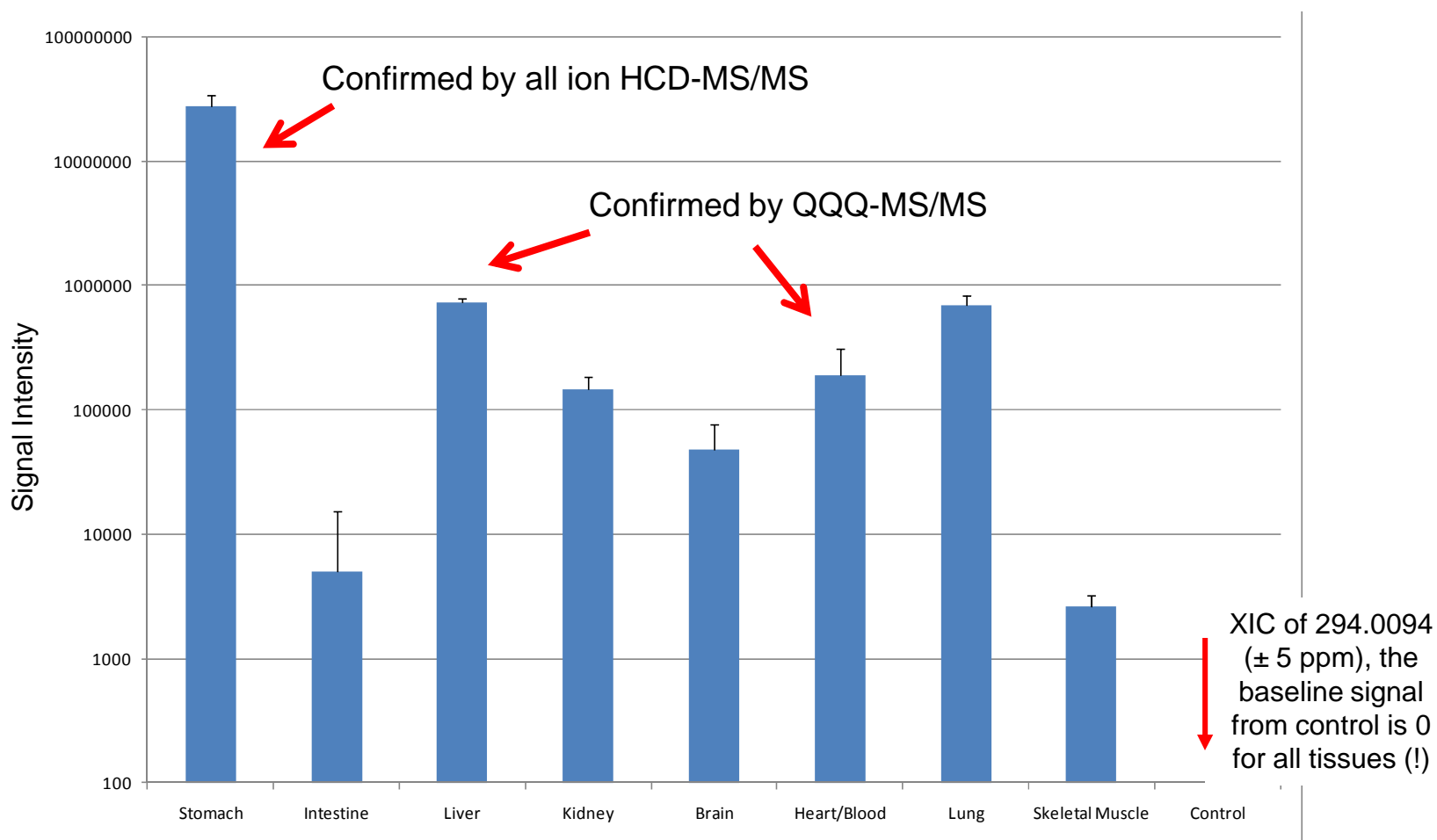
LESA MS Detection of Diclofenac Hydroxy Metabolites : 5500 Kidney Tissue: Positive Ions



Positive ion MS/MS provides more structural characterization

Summary Drug Tissue Distribution Analysis

– Diclofenac 15 min after P.O. 10 mg/kg Diclofenac –



Average of n=4 locations on tissue and SD

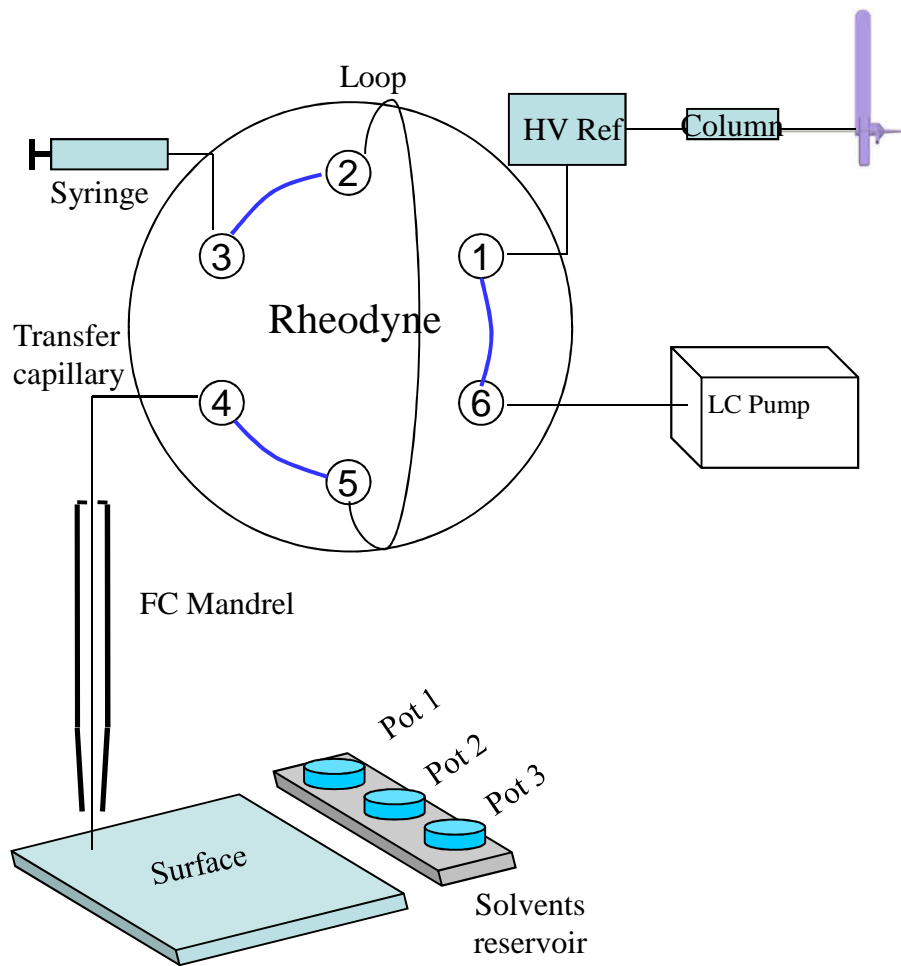
Detection of Isobaric Metabolites in Tissue

Hydroxy-Diclofenac in Kidney 15 min P.O 10 mg/kg Diclofenac in mouse

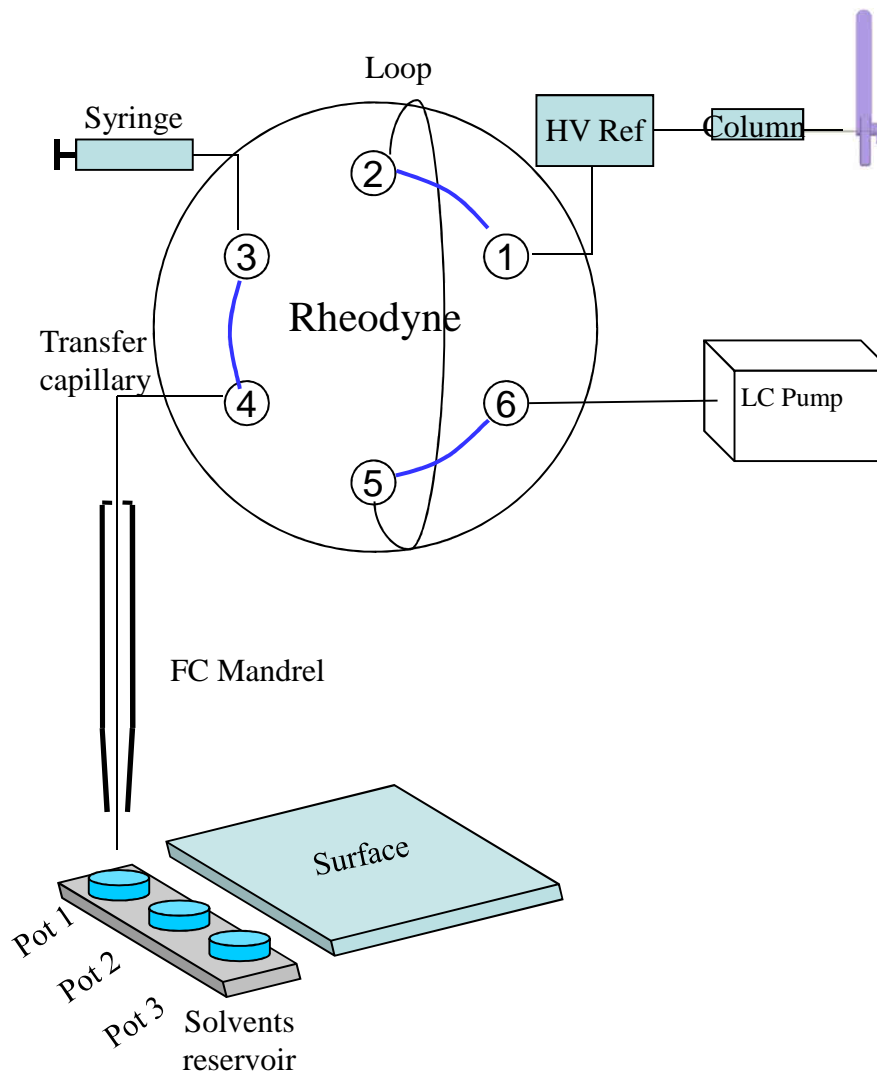
- LESA followed by direct infusion could not differentiate between the two hydroxylated metabolites.
 - Positive ion mode MS3 (QTRAP) produced indistinguishable spectra (data not shown)
- Differentiation could be facilitated by:
 - LC separation of LESA extract.
 - Differential ion mobility employing solvent gas additives

Set Up for LESA Directly Coupled with nanoLC/MS

Pos 1



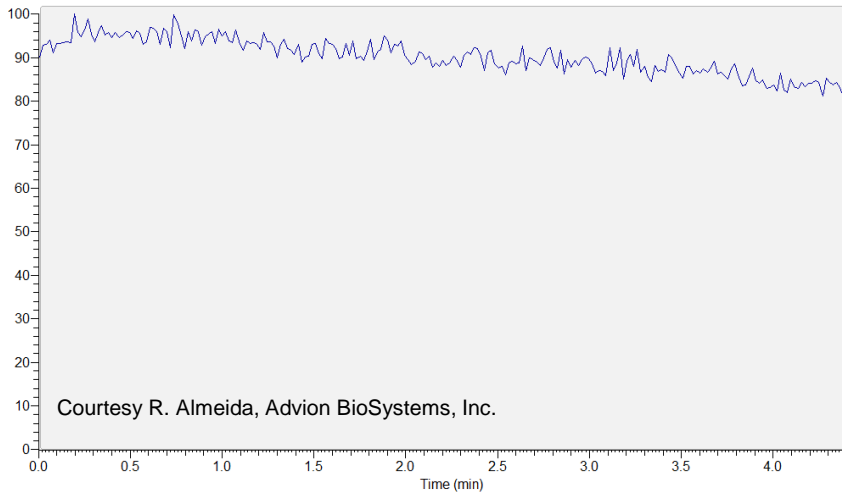
Pos 2



Comparison LESA vs. LESA-nanoLC

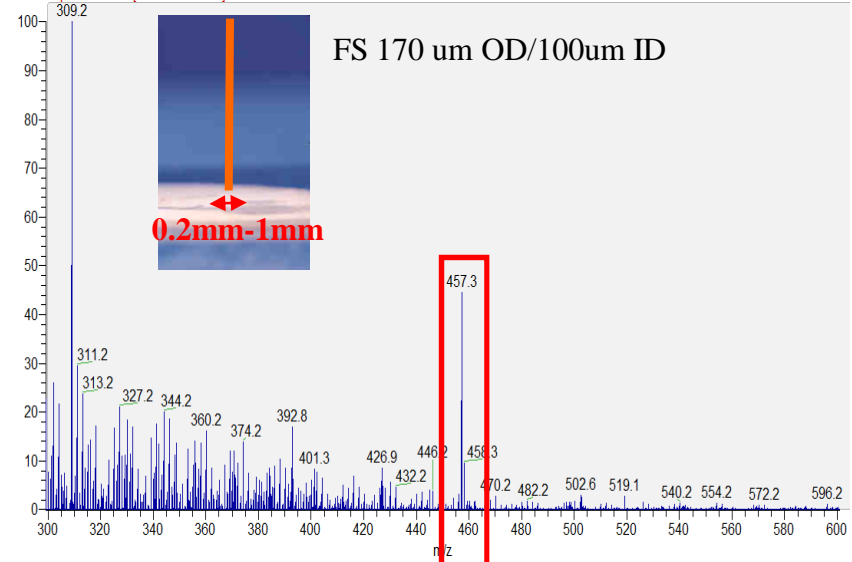
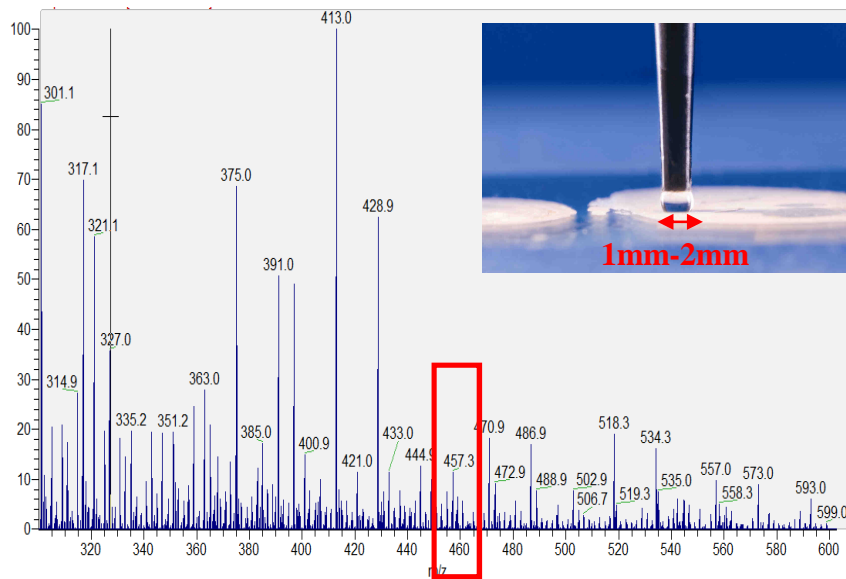
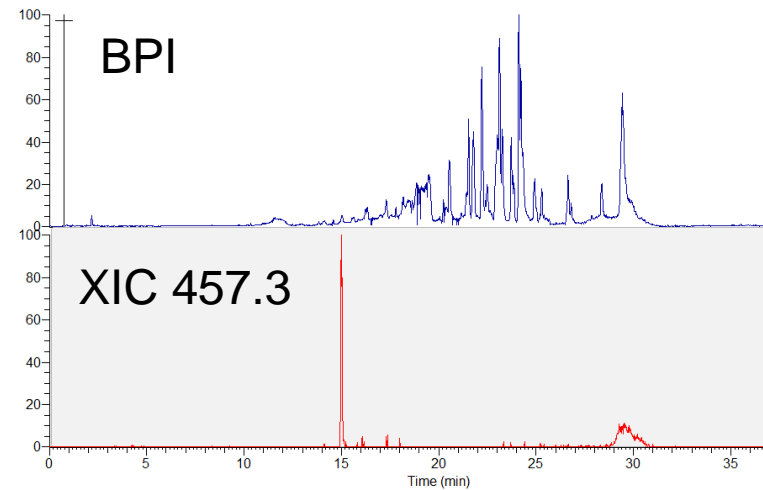
LESA

1 uL → 5 min at 200nL/min



LESA-nanoLC

1 uL injected on column 35 min Gradient



Summary

- Diclofenac can be determined by electrospray-MS and MS/MS in thin whole body sections using LESA-MS
 - These techniques are often complementary
- LESA with positive and negative ion analysis modes provides optimal capabilities
- LESA-MS for Diclofenac is most sensitive in negative ion mode and MS analysis
 - High resolution/accurate mass accuracy provides high selectivity
- Parent drug confirmed by MS/MS experiments
 - Positive ion MS/MS is most useful for confirmation purposes;
 - Confirmation may be provided by:
 - HCD-all ion MS/MS
 - QQQ-MS/MS
 - this mode is more sensitive
- Two Different types of Diclofenac Metabolites were detected
 - beta-D-Glucuronides in Liver
 - Hydroxy-Diclofenac in Kidney

Conclusions/Future Work

- **LESA-MS can provide helpful drug and metabolite distribution information in tissue sections**
 - **LESA-nanoLC/MS could provide additional analytical utility**
- **Implementation of differential mobility spectrometry(DMS) between ionization and MS stages could facilitate isobaric metabolite differentiation**
- **Comparable MALDI imaging data for diclofenac and its metabolites has not been found in the literature**

Acknowledgements



Advion BioSciences Headquarters in Ithaca, NY

More LESA @ ASMS 2011:

MP 037 – Cox et al.
MP 258 – Himmelsbach et al.
MP 269 – Almeida et al.
TOF 2:30 – Wagner et al.
TOF 4:10 – Eikel et al.
WOF 9:50 – Parson et al.
WP 413 – Blatherwick et al.
ThP 285 – Taguchi et al.
ThP 379 – Porta et al.
ThP 380 – Prosser et al.

... or at www.advion.com

We would like to thank Thermo Fisher Scientific and AB/Sciex and for the generous loan of an Exactive Benchtop Orbitrap and QTrap 5500 mass spectrometer, respectively.