The Development of Single-Use, Biocompatible SPME Fibers for Solvent Desorption

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Overview of Presentation

- Goals for SPME-LC Fibers
- Fiber coating properties and process
- Description of fiber and device
- Applications
  - Drugs out of buffer & plasma
  - *In-vivo* application
- Interface with DESI
- Conclusions

Advantages and Disadvantages of Current SPME Fibers

Advantages – Useful for GC analysis

1. Good thermal stability with low background
2. Can extract volatile and semi-volatile compounds, from air, water and a variety of solid matrices
3. Can be immersed in water or exposed to headspace
4. Fully automated with GC instruments
5. Over 1800 published articles using SPME
6. Derivatizing agents can be applied to fibers

Disadvantages – Not very useful for HPLC analysis

1. PDMS based fibers can swell in nonpolar solvents
2. Polyacrylate and PEG can swell in polar organic solvents and water
3. Difficult to desorb nonvolatile analytes with solvents
4. No direct automated interface with LC instruments

Goals of Development of SPME Fibers for Solvent Desorption

The Coating:

1. Fiber coating must durable and reproducible
2. Fiber coating must not swell in water or organic solvents
3. Bonded silica such as that used in HPLC chosen for coating

The Binder:

1. Binder should not affect uptake of analytes
2. Binder should be biocompatible
    a. Resists large macromolecules
    b. Can be with in-vivo type experiments without harm to organism

Device needs to be low cost for single use analysis

Fiber Coating - Process and Properties

1. Silica particles (3 µm or 5 µm) are embedded in a biocompatible proprietary binder
2. Particles are coated on a durable, flexible 200 µm metal fiber using an automated coating process (45 µm coating thickness variability 1-3%).

Findings

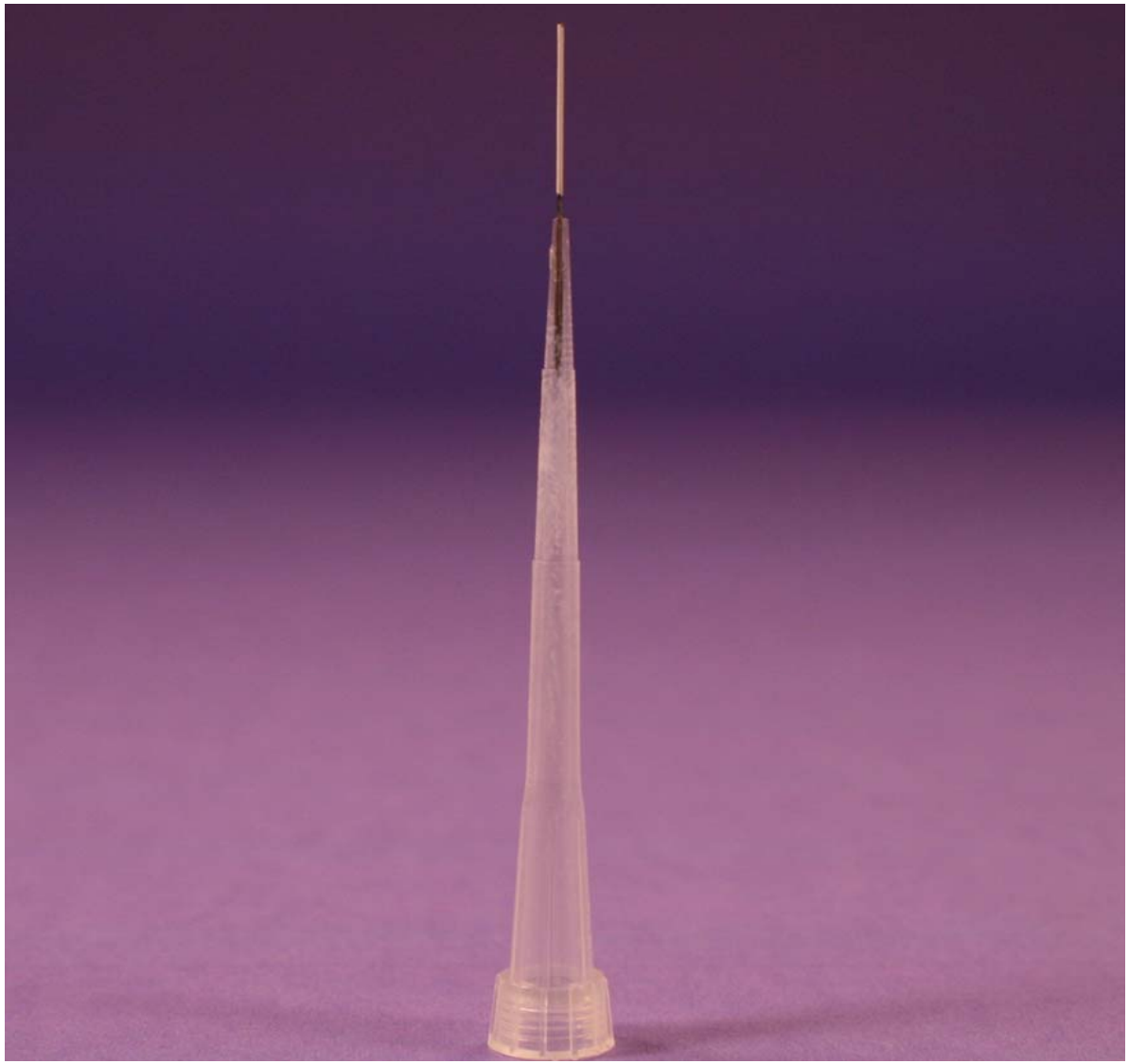
1. Binder is inert and does not swell in water or organic solvents
2. Binder does not impede extraction of small molecules
3. Binder repels proteins and large macromolecules

Swelling of Fiber Coating Upon Solvent Exposure

Fibers soaked for 15 min in each solvent

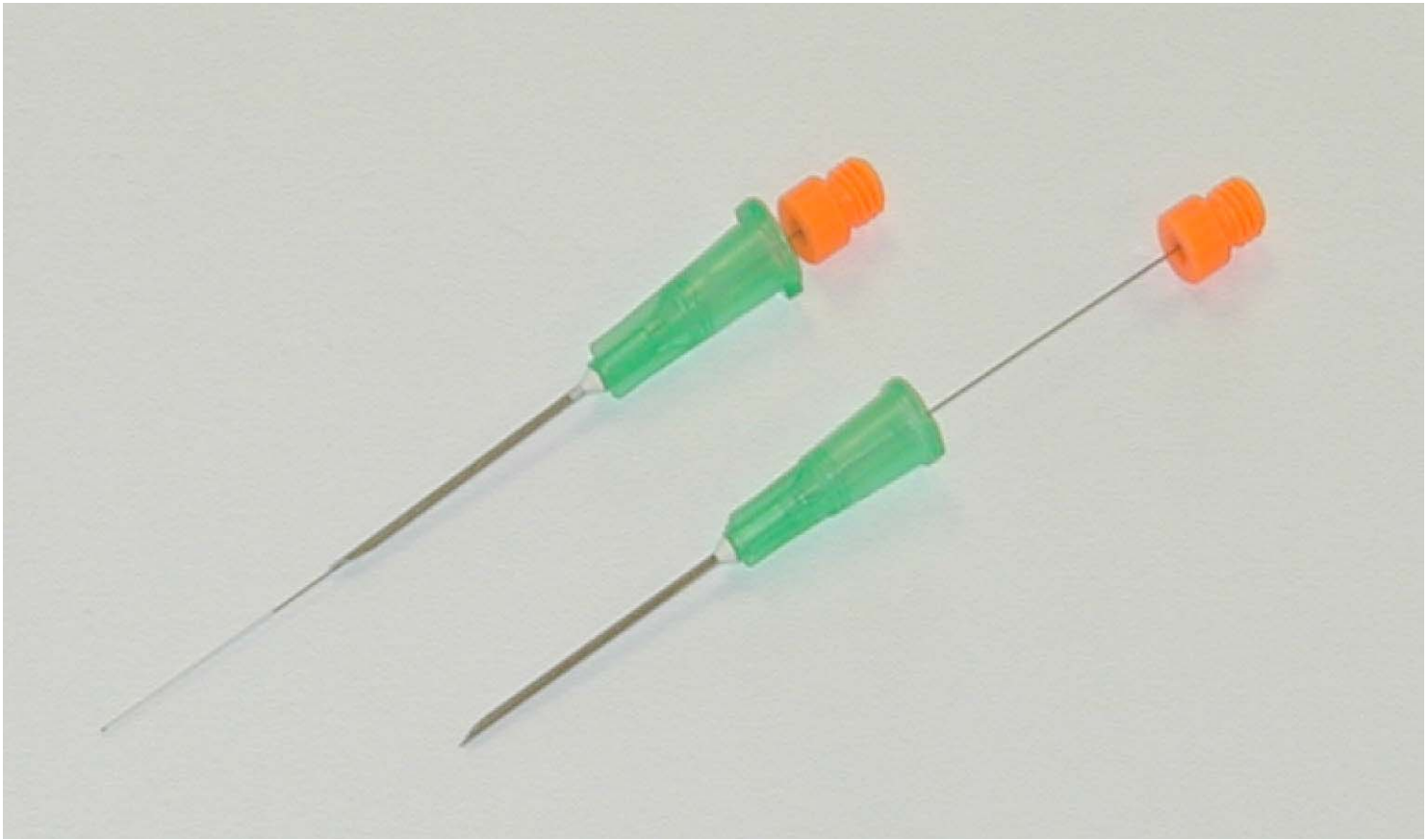
	Bonded Silica Fiber Coating Thickness µm			Carbowax®-TPR Coating Thickness µm		
Solvent	**No Solvent**	**15 Min. in Solvent**	**Difference**	**No Solvent**	**15 Min. in Solvent**	**Difference**
Water	44	44	0	50	60	10
Acetonitrile	44	44	0	50	51	1
Methanol	44	44	0	50	61	11
Dichloromethane	44	44	0	50	52	2
Hexane	44	44	0	50	50	0
Acetone	44	44	0	50	50	0
Water:ACN	44	44	0	50	70	20
Water:MeOH	44	44	0	50	68	18

Fiber Tip for HPLC Analysis

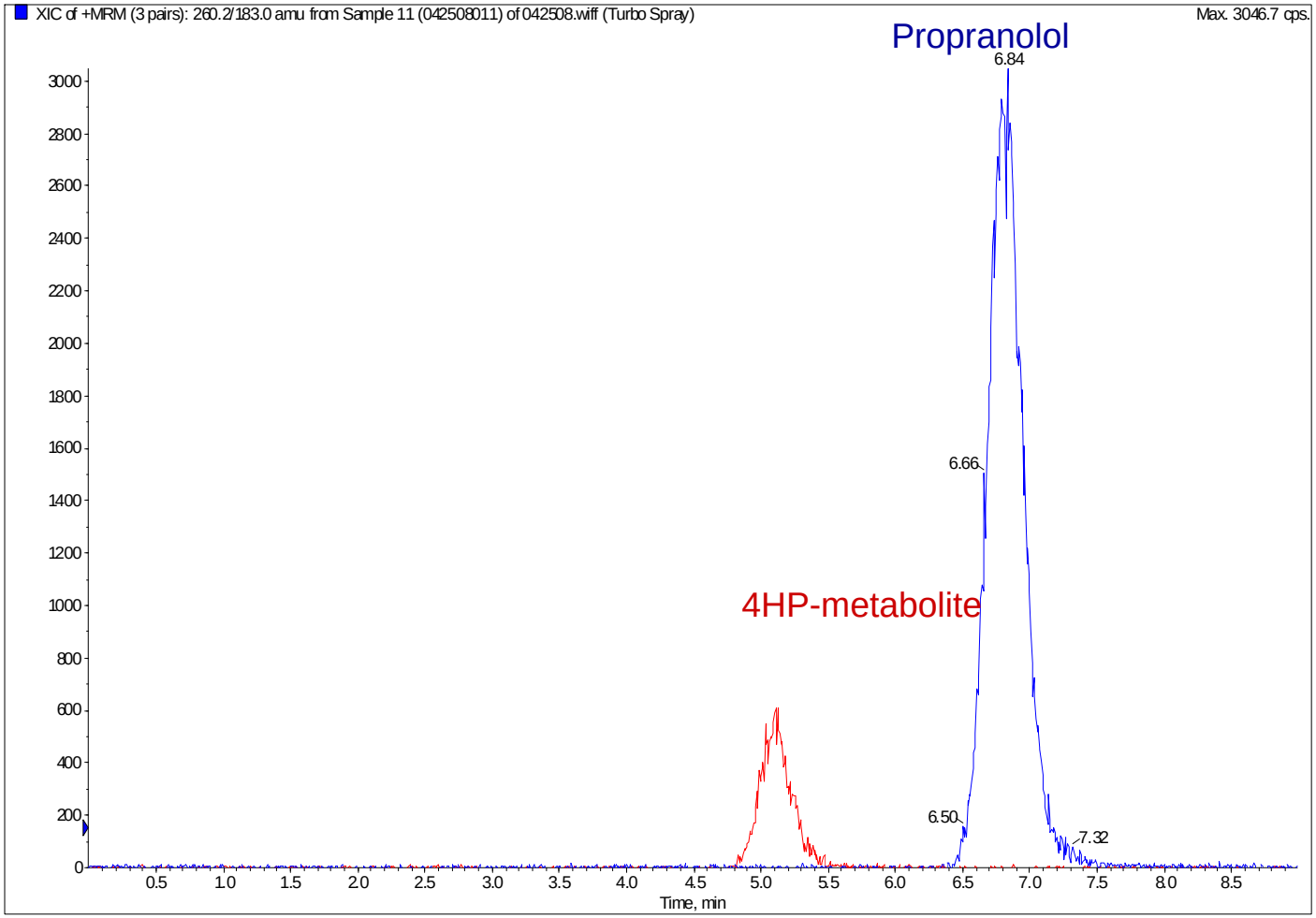


Fiber Pipette Tips

Single Use Biocompatible Fiber Probes for *in-vivo* Analysis



HILIC Separation of Propranolol and 4-hydroxypropranolol (4-HP) Metabolite



LC-MS-MS Conditions

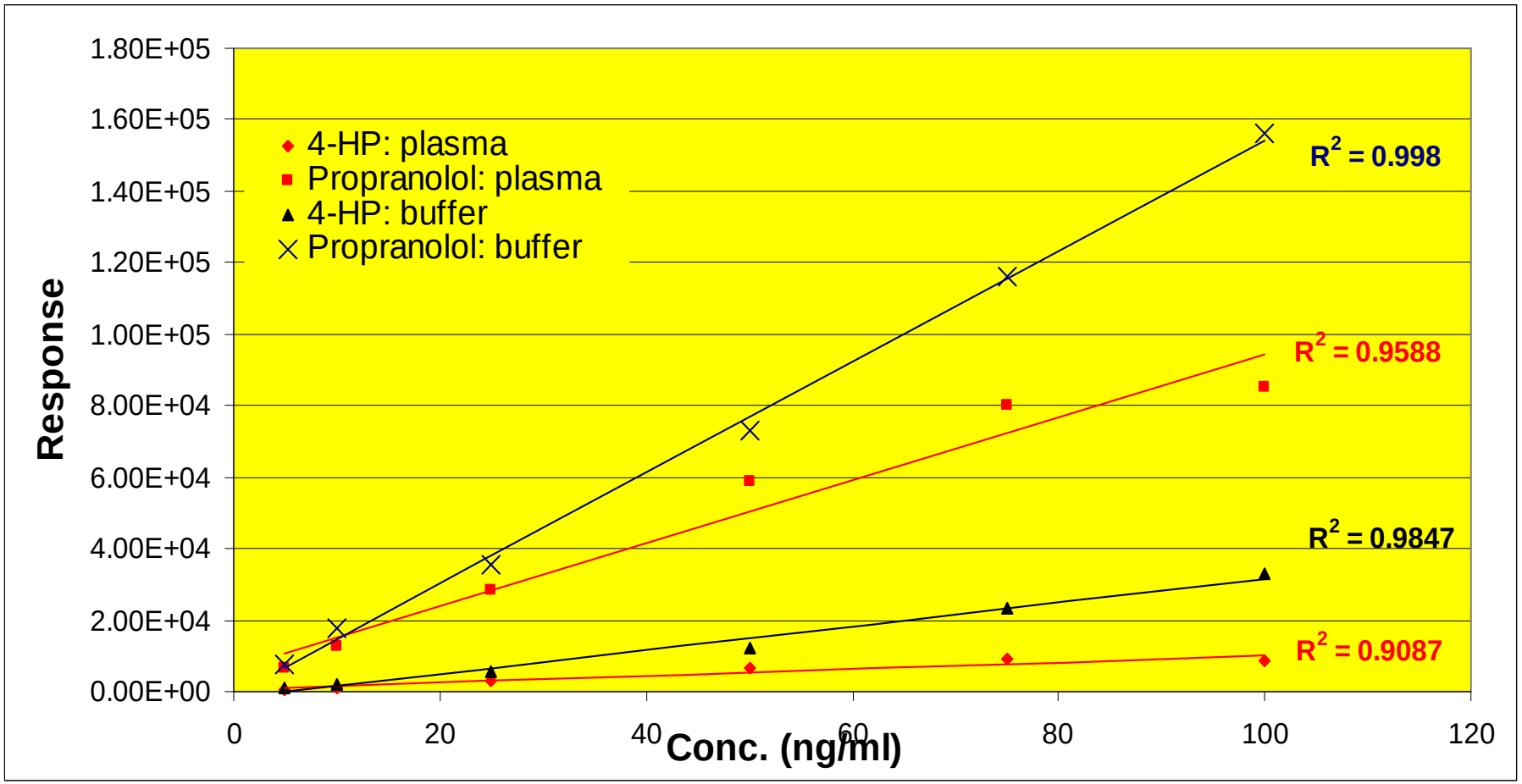
Instrument	Applied Biosystems 3200QT
Column	Discovery® HS F5; 5 cm x 2.1 mm, 3 µm
Mobile Phase	2 mM ammonium formate in 90:10 acetonitrile:water
Flow	200 µL/min.
Temperature	35 ° C
Injection Volume	5.0 µL
Source Conditions	Turbo ion spray ESI +, MRM
Q1 Mass (amu)	Propranolol: 260.21 4-hydroxypropranolol: 276.21
Q3 Mass (amu)	Propranolol: 183.00 4-hydroxypropranolol: 173.10
Dwell time	150 msec

SPME Extraction Conditions

Fiber Type	C18 Fiber, 45 µm coating thickness
Sample	500 µL and 100 µL, spiked phosphate buffer ,and rat plasma adjusted to pH 4.0 with 25%H_3PO_4
Fiber Conditioning	15 min. in methanol
Fiber Equilibration	15 min. in water
Extraction	10 min., static
Desorption	60 min. in 100 µL 13mM NH_4OAc in 90:10 ACN:H_2O

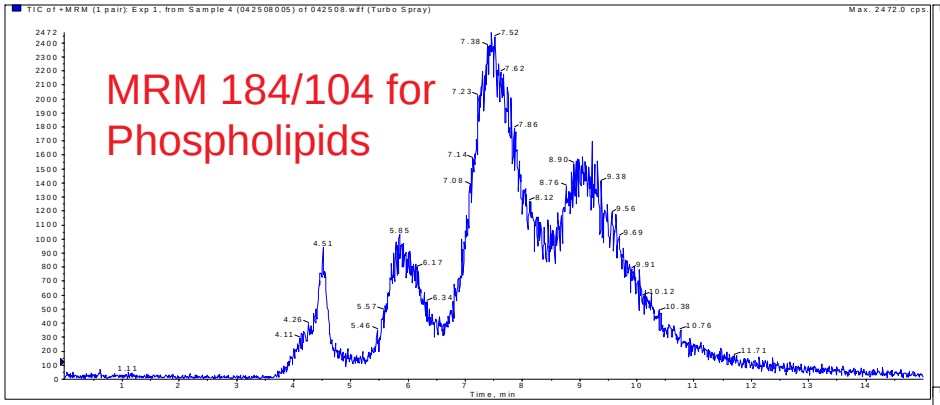
Prior to each extraction, fibers are first condition in methanol followed by equilibration in water. Phosphate buffer contained 0.8% NaCl to mimic concentrations consistent of blood plasma.

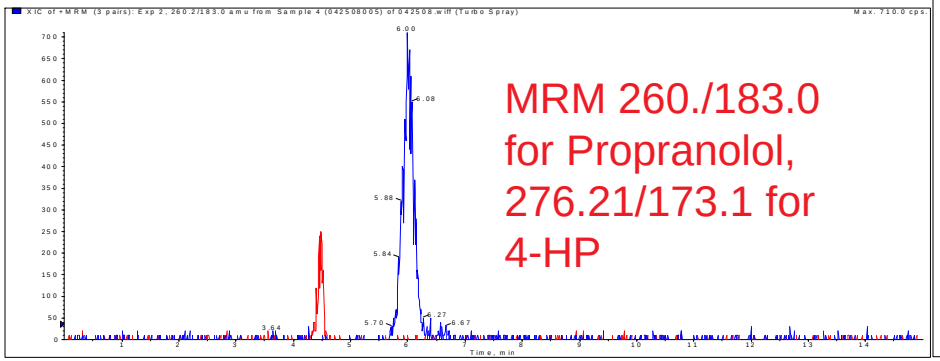
Linearity of Extractions of Propranolol and 4-HP from 100 µL Samples



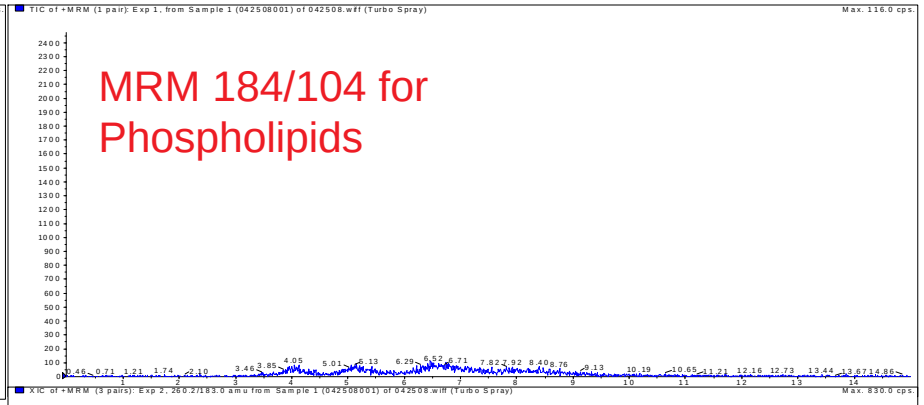
LC-MS Analysis of Drugs in Plasma: Comparison of SPME Extraction to Direct Injection on the Matrix Background and Detection of the Drugs

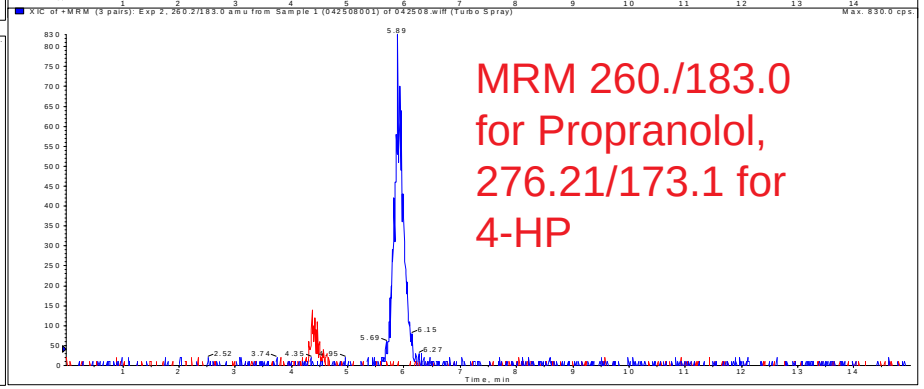
Direct Injection after protein precipitation



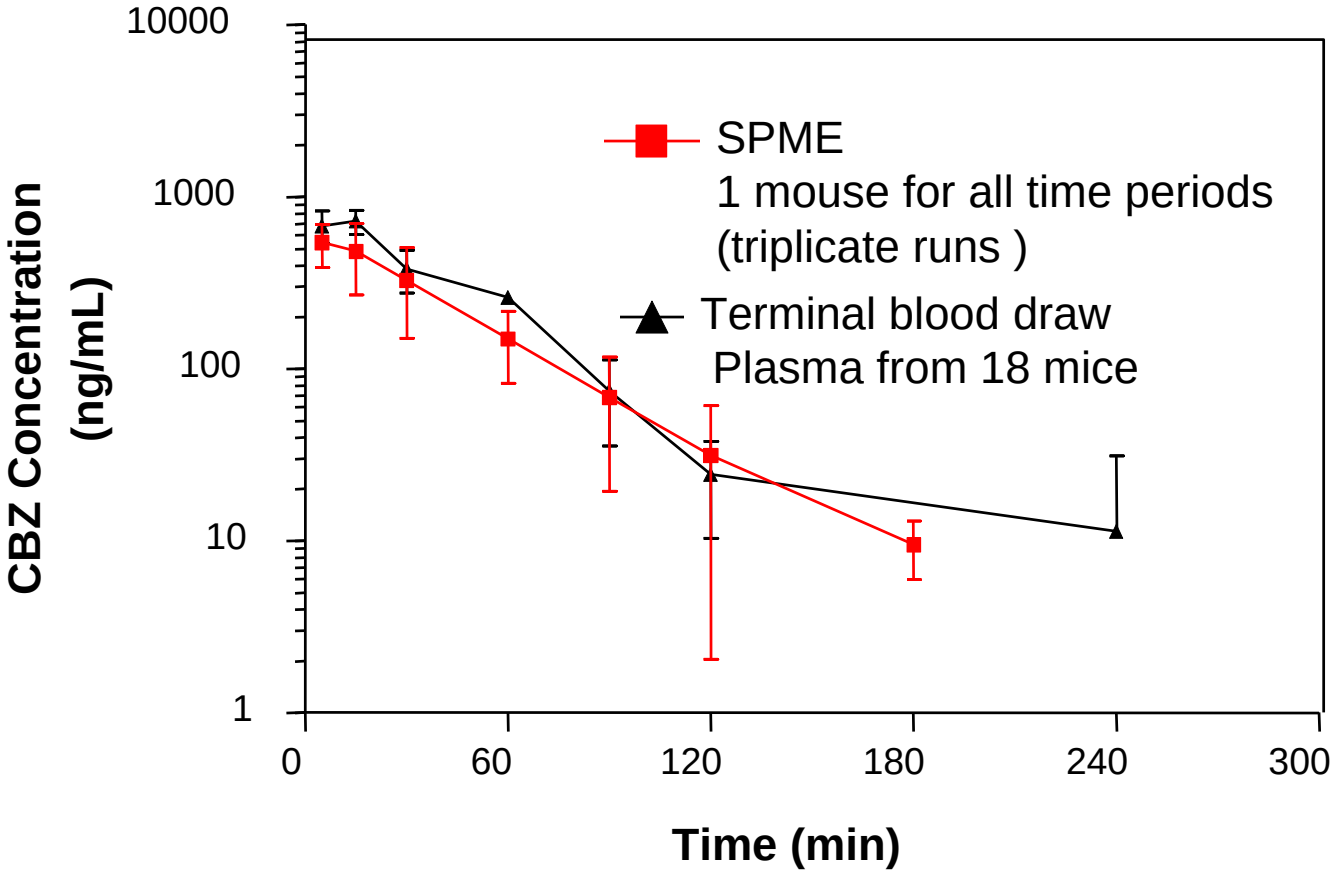


SPME Extraction



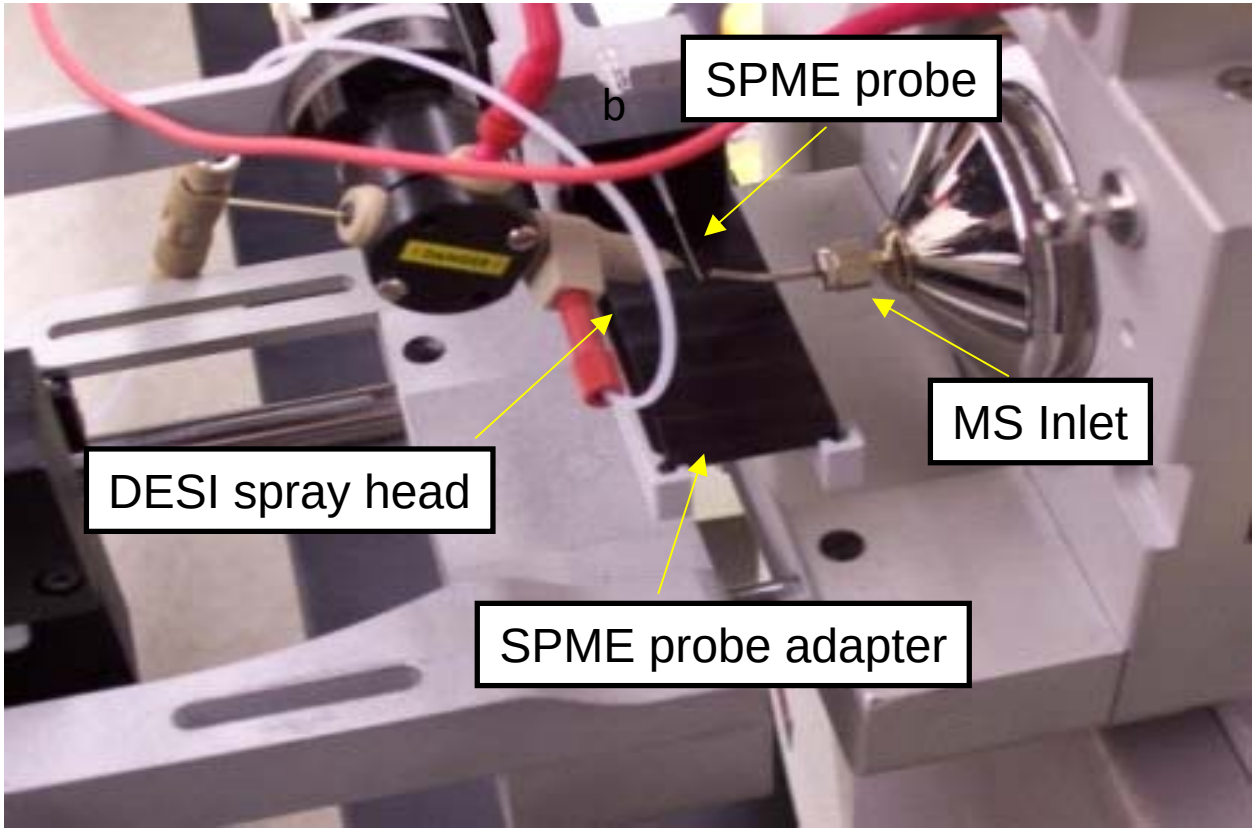


Comparison of SPME *in-vivo* Pharmokinetics (PK) Study of Carbamazepine (CBZ) from Mice Whole Blood to Extracts of Plasma Removed from Mice



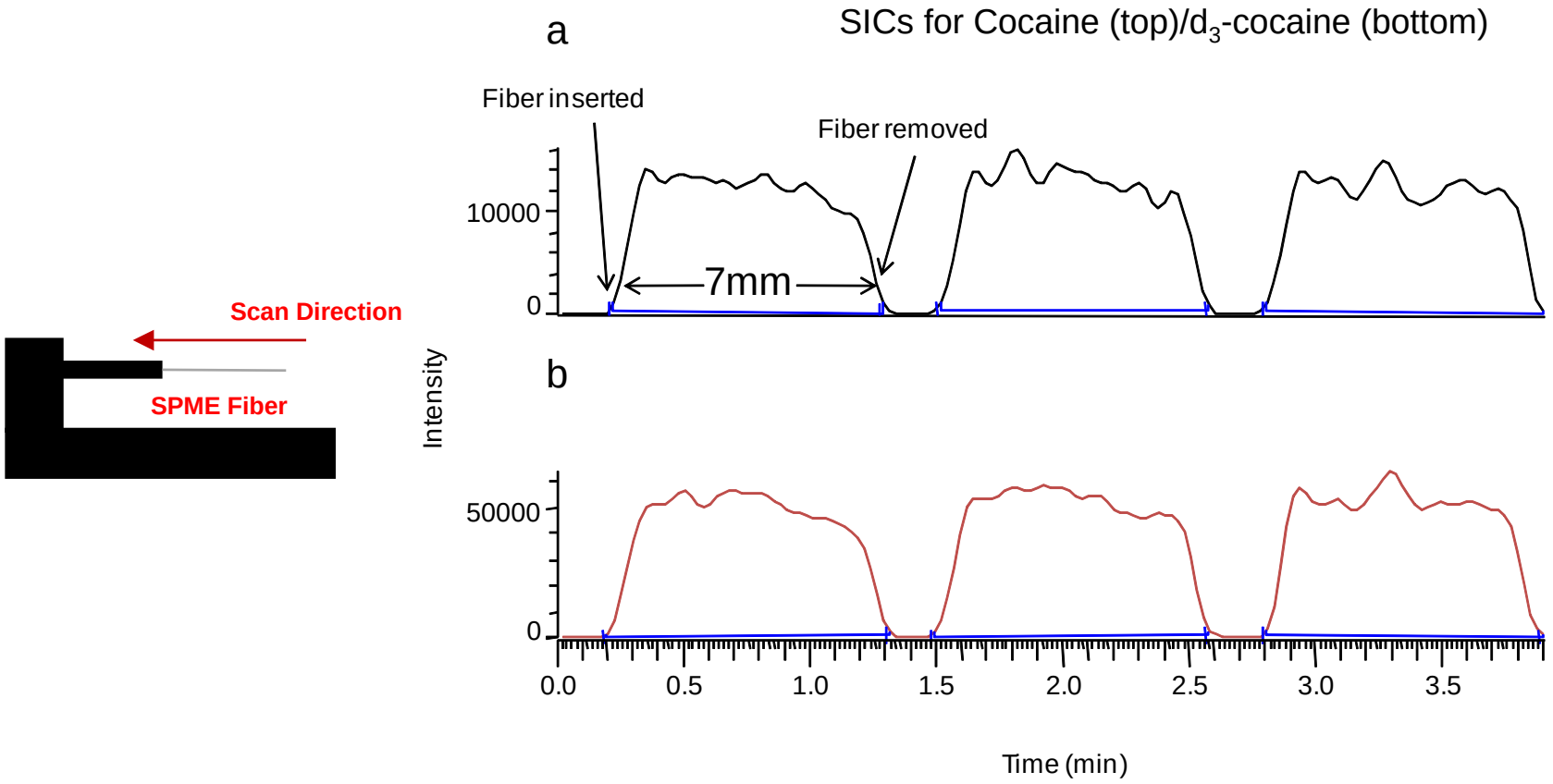
Slide Courtesy of Ines de Lannoy-NoAb BioDiscoveries

Example of Direct Desorption of Conventional SPME Fibers by DESI



Courtesy of Joseph Kennedy of Prosolia

The Result of Scanning is Called a Chronogram



Courtesy of Joseph Kennedy of Prosolia

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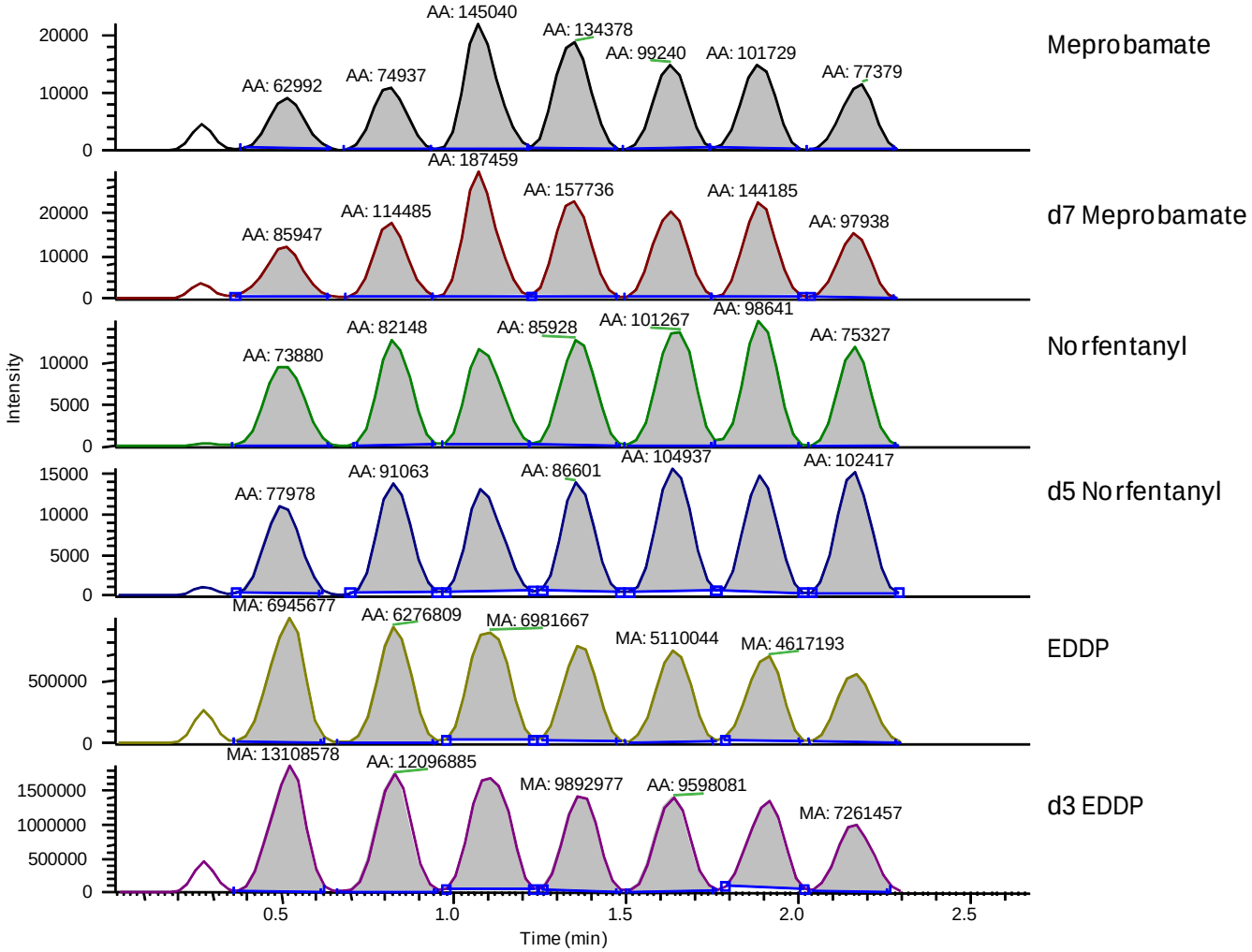
Chronogram:
Analysis of a single fiber, scanned multiple times



Courtesy of Joseph Kennedy of Prosolia

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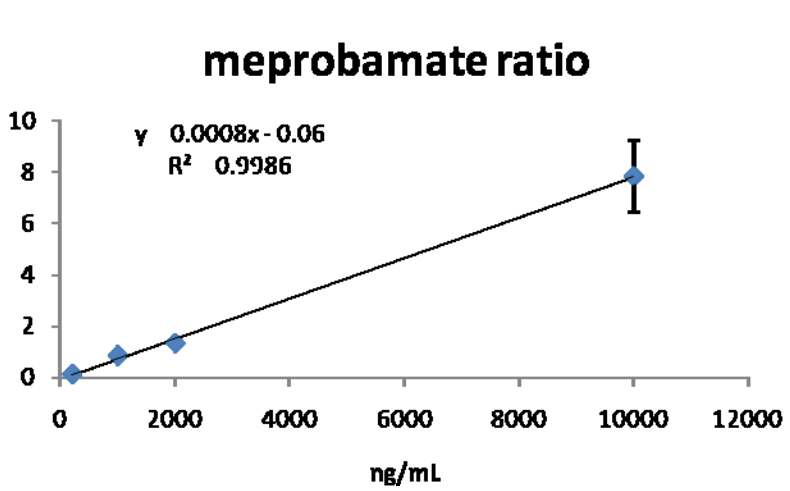
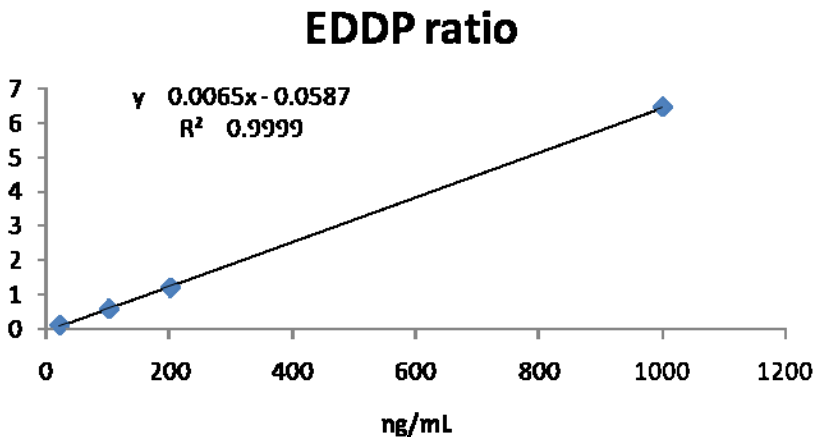
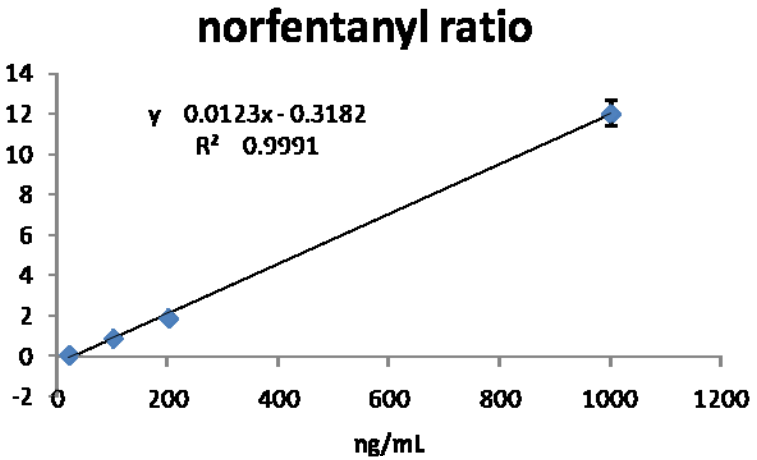
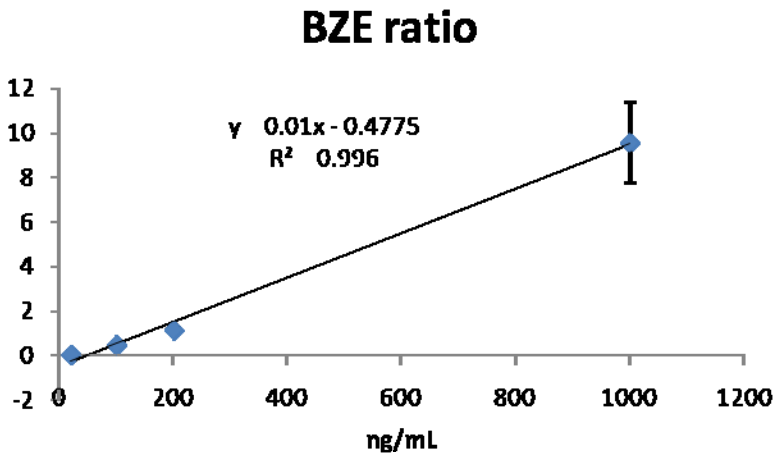
Calibration Curves







Error bars represent 1 std dev



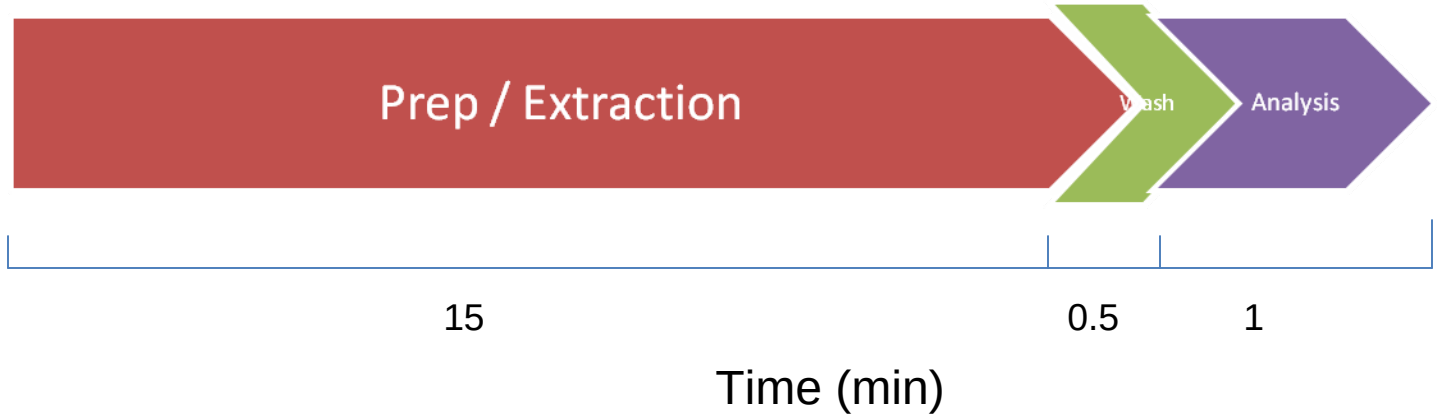
Courtesy of Joseph Kennedy of Prosolia

SPME / DESI-MS Analysis Time Line

Procedure:

Pipette labeled internal standard (200 ng/mL) into Urine,
Batch extractions for 15 minutes (optimize),
Wash with water,
Analyze fiber with DESI-MS/MS 1 min

(optimize)



Courtesy of Joseph Kennedy of Prosolia

Conclusions

- SPME fiber coatings have been specifically designed for HPLC use and bio-applications
- Fiber does not swell in water and/or solvents
- Fiber is biocompatible
- Fiber is durable and reproducible
- Fiber probe is suitable for in-vivo and in-vitro applications
- Fiber can be coupled with DESI
- Different coatings are being evaluated

Acknowledgements

Ines de Lannoy – In vivo applications
Joseph Kennedy – DESI applications

Thank you!

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