

# Solid Phase Micro Extraction (SPME) of Opiates from Urine: Coupling SPME and DESI-MS/MS Detection



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# Outline

- Solid Phase Micro Extraction (SPME)
- Direct ElectroSpray Ionization (DESI)
- Proof of Concept Application – Analysis of Opiates in Urine using SPME/DESI
- In-vivo application of SPME
- Summary and challenge

# Solid Phase Microextraction (SPME)

Holder assemblies (manual, autosampler, robots)

Coated fibers (adsorbent and absorbent)

Janusz Pawliszyn, Univ. of Waterloo; unique and proprietary to Supelco

Economical enrichment technique mainly for trace analysis

## Features:

- Very limited or no use of solvents
- All types of samples & matrixes
- Direct immersion or headspace
- Designs for manual, autosamplers and robots

## Benefits:

- Economical
- Highly consistent, quantifiable results
- Portable (field use) and reusable



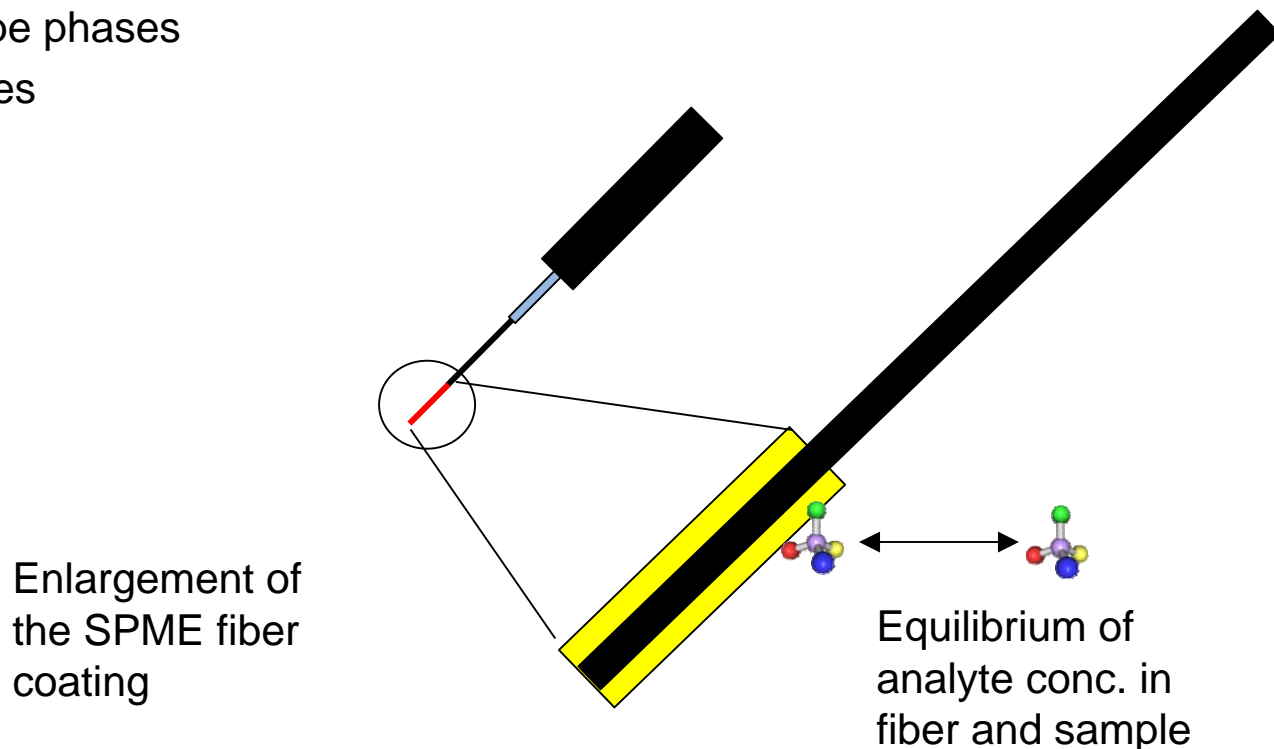
# SPME Fiber Coating: The Business End

Not an exhaustive extraction technique

An equilibrium is set up between analytes dissolved in the sample (solution or gas phase) and in the liquid coating on the fiber.

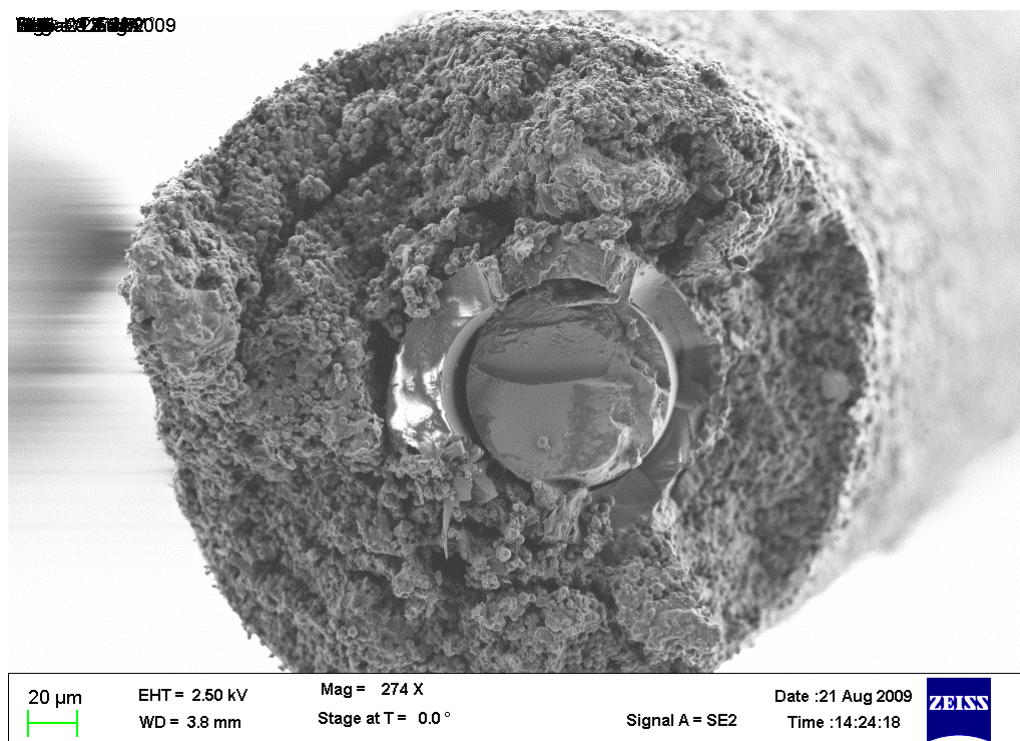
The fiber coating consists of:

- GC-type phases
- Particles



# PDMS-DVB Fiber SEM

Cross section of the PDMS-DVB fiber. The center is a fused silica core, surrounded by a Stableflex core. The 3-5 $\mu$ m DVB particles are suspended in PDMS and layered over the cores. 275x magnification.



Photomicrograph of SPME fiber provided by Prof. Dan Armstrong, U. Texas Arlington

# Distribution Constant

Concentration of analyte in stationary phase compared to concentration of analyte in solution:

$$K = n_s / V_1 C_2^\circ$$

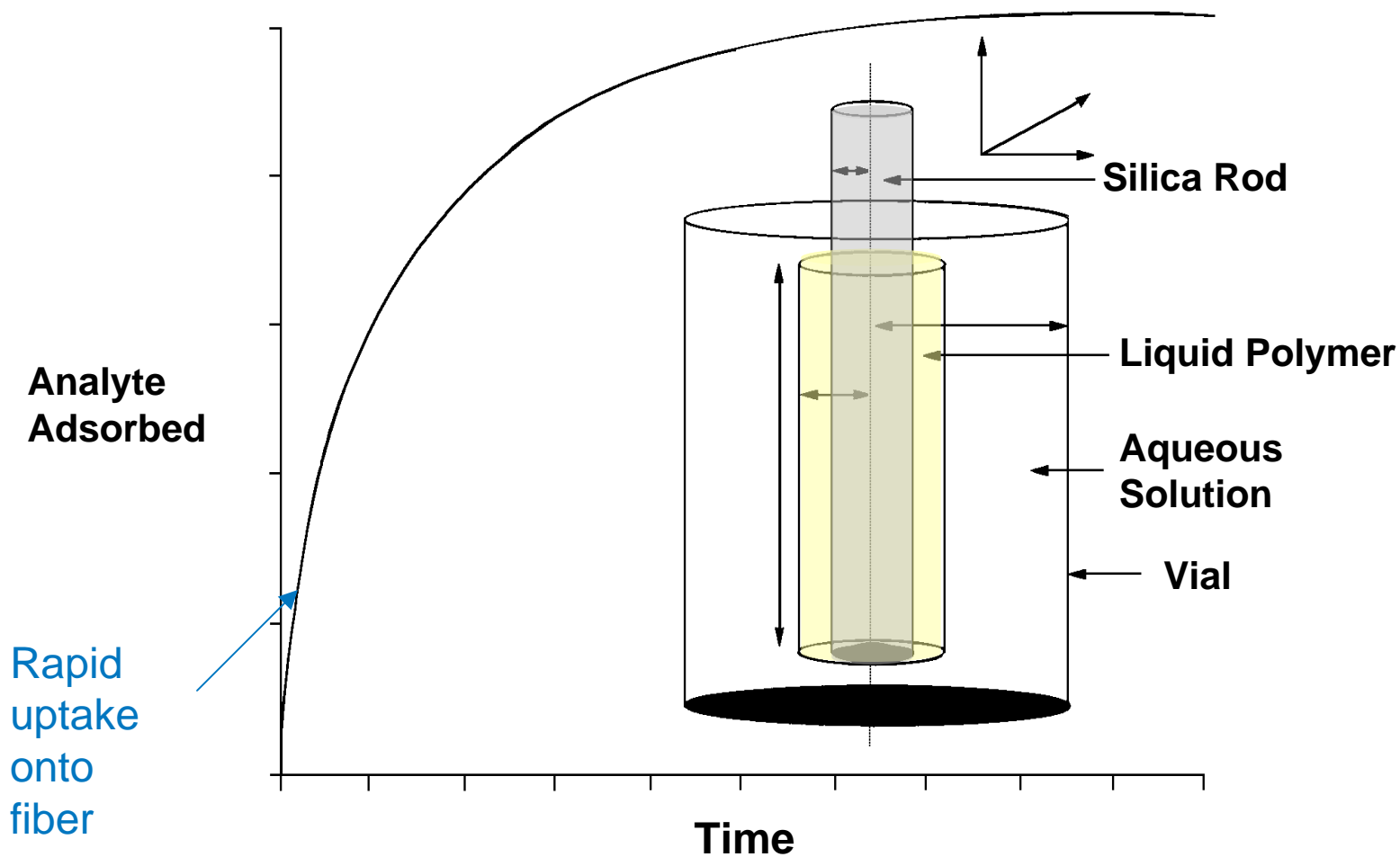
K = Distribution constant

$n_s$  = Moles of analyte in stationary phase

$V_1$  = Volume of stationary phase

$C_2^\circ$  = Final analyte concentration in sample

# Adsorption Mechanism for SPME



# “Dials” to Turn in SPME Methods

## Device

Type of coating (polarity)  
Coating thickness

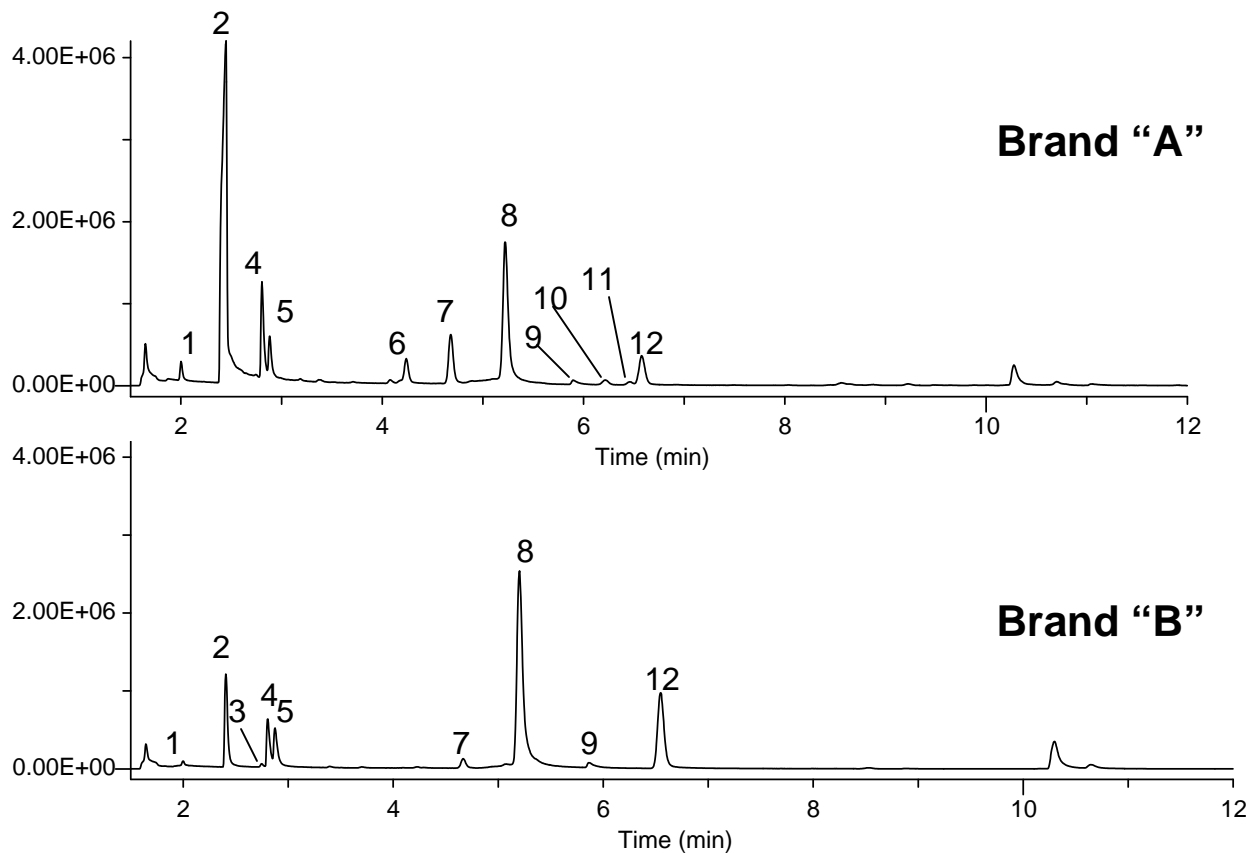
## Sample

Headspace vs. direct immersion extraction  
Ionic strength, pH, polarity of sample solution  
Stirring (sample) & agitation (fiber)  
Extraction time  
Extraction temperature





# Residual Solvents in Commercial Ibuprofen



1. Acetaldehyde
2. Ethanol
3. Acetonitrile
4. Acetone
5. 2-Propanol
6. 2-Methylpentane
7. 3-Methyl pentane
8. Hexane
9. Ethyl acetate
10. 2,2-Dimethylpentane
11. 2,4-Dimethylpentane
12. Methylcyclopentane

# Fiber Pipette Design and Use



**Classically designed for thermal desorption  
– GC-GC/MS**

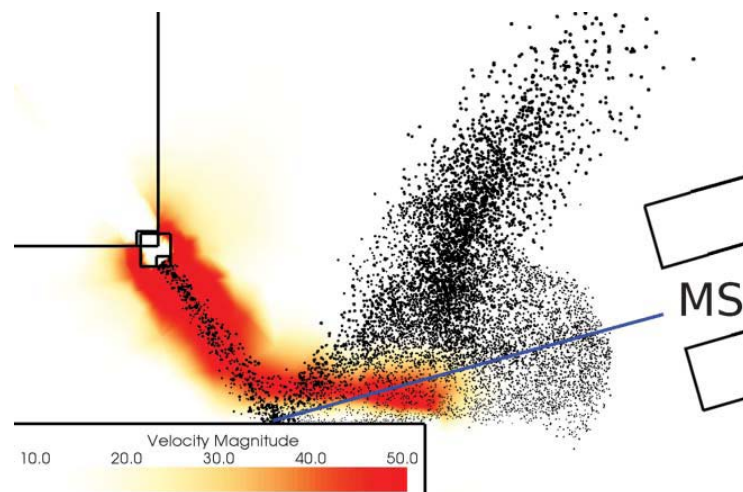
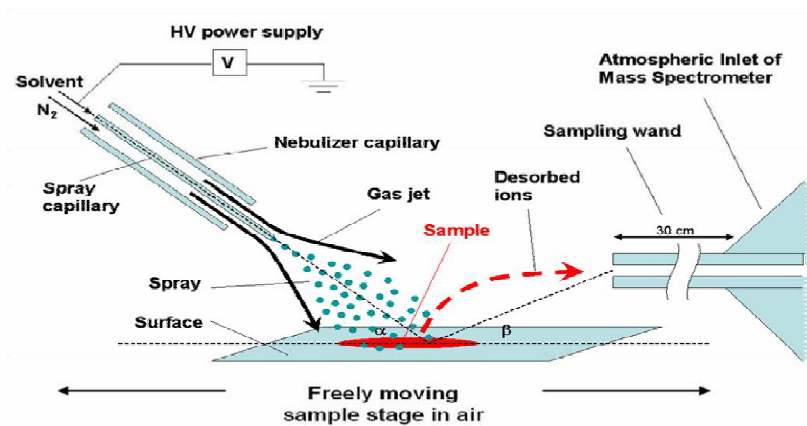
## **New Compelling Designs and Features**

- SPME fiber coatings for direct bioanalytical-applications
- Low protein adsorption
- Fiber does not swell in water and/or solvents
- Many different chemistries are being developed
- Favorable kinetics and capacity
- Suitable for in-vivo and in-vitro applications
- Fiber can be coupled with DESI-MS and other MS systems



# Desorption Electrospray Ionization

Direct sample analysis in the open air



Cooks et al. first published DESI in *Science* (Mass Spectrometry Sampling Under Ambient Conditions with Desorption Electrospray Ionization, *Science*, 2004, 306, 471-473).

A. Costa, R. G. Cooks, *Chem. Comm.* 2007, 3915-3917

DESI uses high velocity charged droplets to desorb and ionize analytes in the sample for analysis in a mass spectrometer.

System and method for desorption electrospray ionization, US patent no. 7337960.

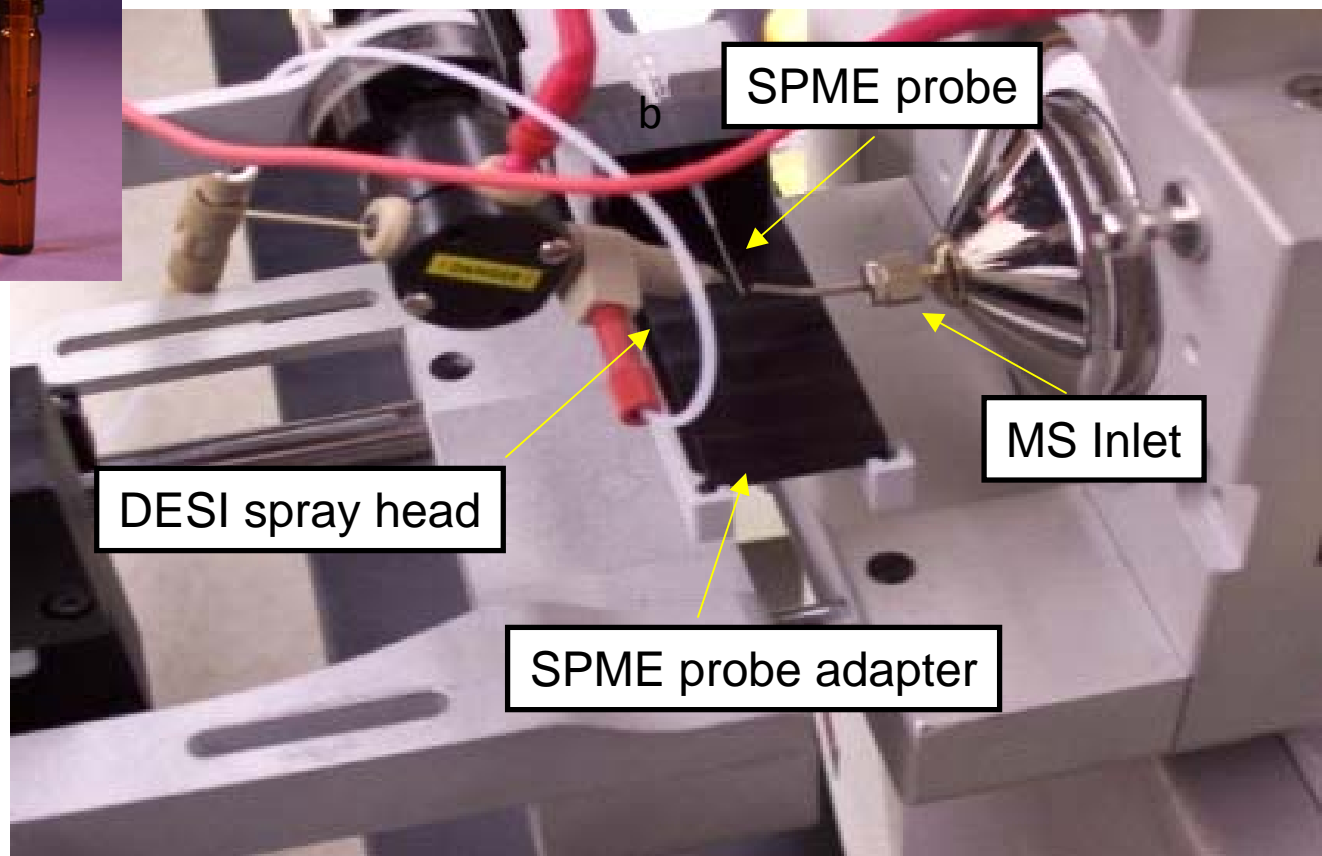
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# SPME-DESI-MS (MS/MS) Approach for Drugs and Metabolites in Biological Fluids

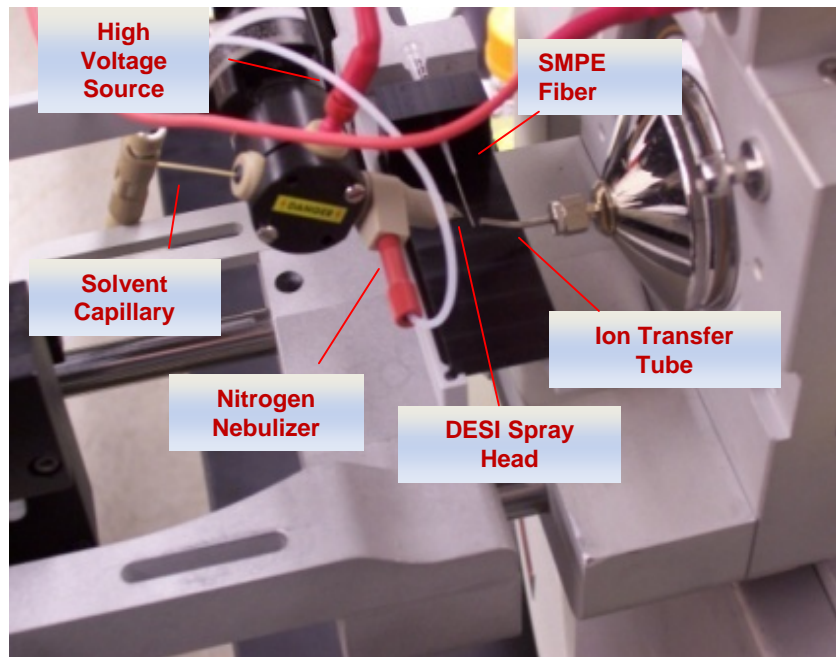
## Compelling features

- Offers minimal sample handling, including sample transfers
- Combines sampling and sample preparation into one step
- Analytes are 'stored' in solid-phase – retained for further evaluation
- Possibility to follow-up direct desorption analysis with liquid desorption LC/MS/MS
- Rapid and potentially high throughput analysis

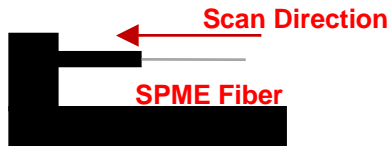
# Example of direct desorption of conventional SPME fibers by DESI



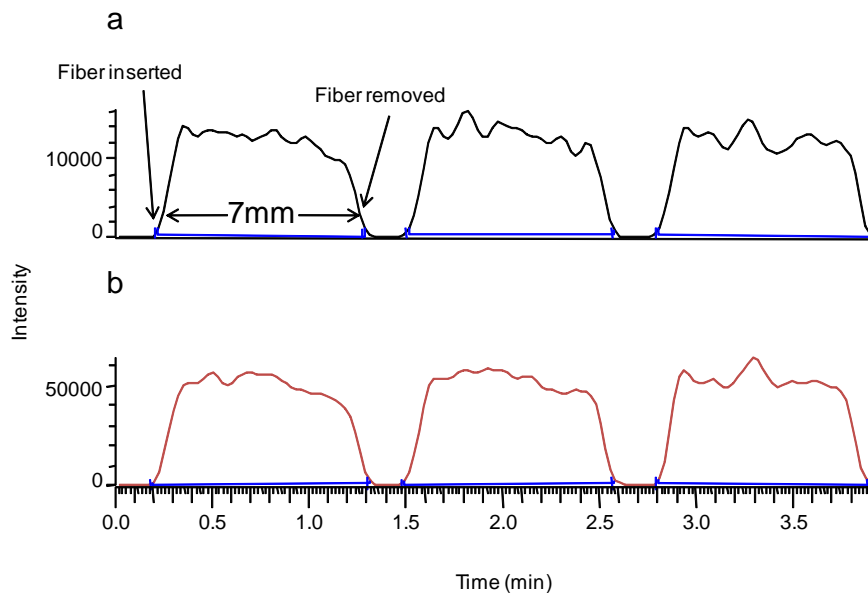
# SPME-DESI-MS/MS



TSQ Quantum Discovery MAX



SICs for Cocaine (top)/d<sub>3</sub>-cocaine (bottom)



Direct insertion of SPME fiber into the spray

- Analogous to so-called “transmission mode” DESI
- Distances are critical; impact distance similar to conventional DESI experiment
- 0.008” SPME fibers coated with silica C18 stationary phase and secured with epoxy inside 20uL eppendorf pipette tip

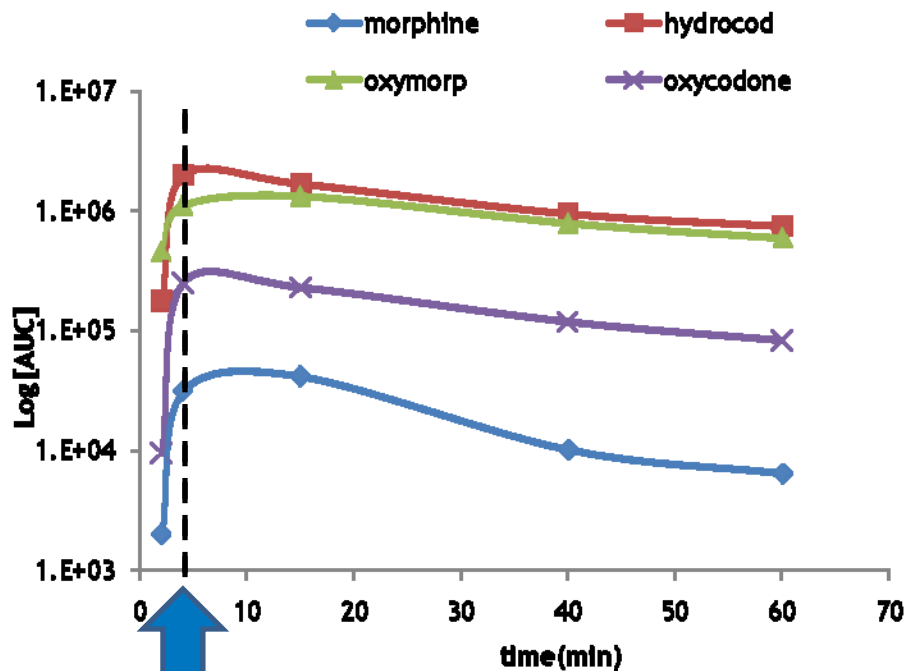
# Analysis of Opiates by SPME-DESI-MS/MS

## Methods:

- Drug free urine samples were spiked with opiates along with stable isotope internal standards.
- Samples were then extracted using recently developed biocompatible SPME fibers coated with functionalized silica particles.
- After extraction, fibers were rinsed with water and secured in a prototype device for positioning the SPME fiber in the DESI spray.
- Analysis by scanning with a 1- D Automated DESI source coupled to a Thermo TSQ Quantum Discovery Max triple quadrupole mass spectrometer
- Analysis was completed in approximately 1 minute.

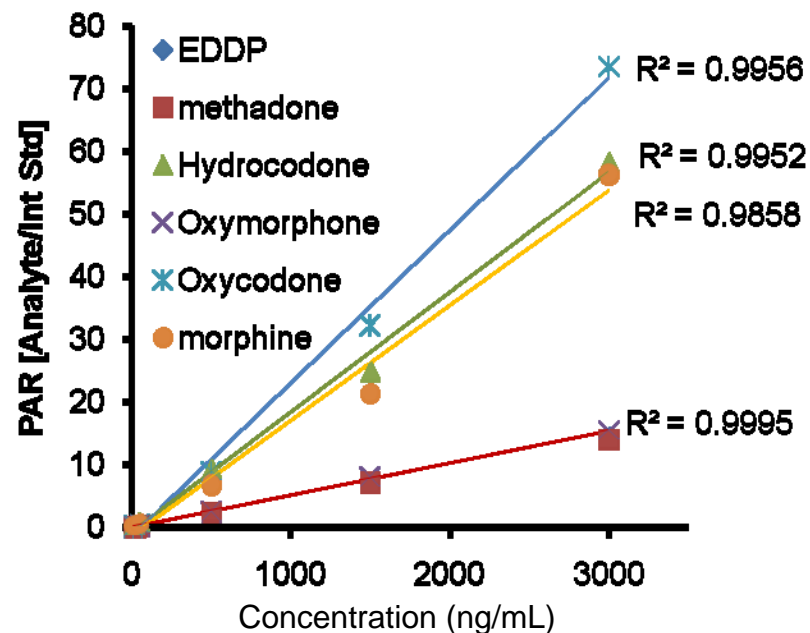
# SPME Extraction Time and Calibration Curve

## Extraction Profile Analyte



5 min extraction in unprocessed urine

## Calibration plot





## Opiate method performance

Analyte	Concentration Range (ng/mL)	R <sup>2</sup>	LLOQ (ng/mL)	CV (%)*	Accuracy (%)
Morphine	10 - 3000	0.9880	36.1	12.7	71-104
Hydrocodone	10 - 3000	0.9952	2.83	9.1	105-115
Oxymorphone	10 - 3000	0.9995	46.8	4.1	89-93
Oxycodone	10 - 3000	0.9956	42.8	20.5	84-106
Methadone	10 - 3000	0.9998	2.77	1.6	87-115
EDDP	10 - 3000	0.9999	0.26	1.2	94-97

Spiked drug-free urine samples

# Drugs of Abuse: Live Patient Samples From AIT

## Methods:

- Raw urine samples were acquired from AIT Laboratories, all samples had been previously been tested by ELISA and standard LC-MS/MS analysis.

Analyte	Range Detected (ng/mL)	SPME DESI-MS/MS	Lab*
Meprobamate	0	0	0
Norfentanyl	0	0	0
EDDP	(13 – 57824)	4	4
Methadone	(18 – 17840)	4	4
Benzoyllecgonine	(74 – 24178)	19	19
Cocaine	(163 – 460)	3	NT
Coca-ethylene	(50 – 158)	2	NT

# SPME-DESI CONCLUSIONS

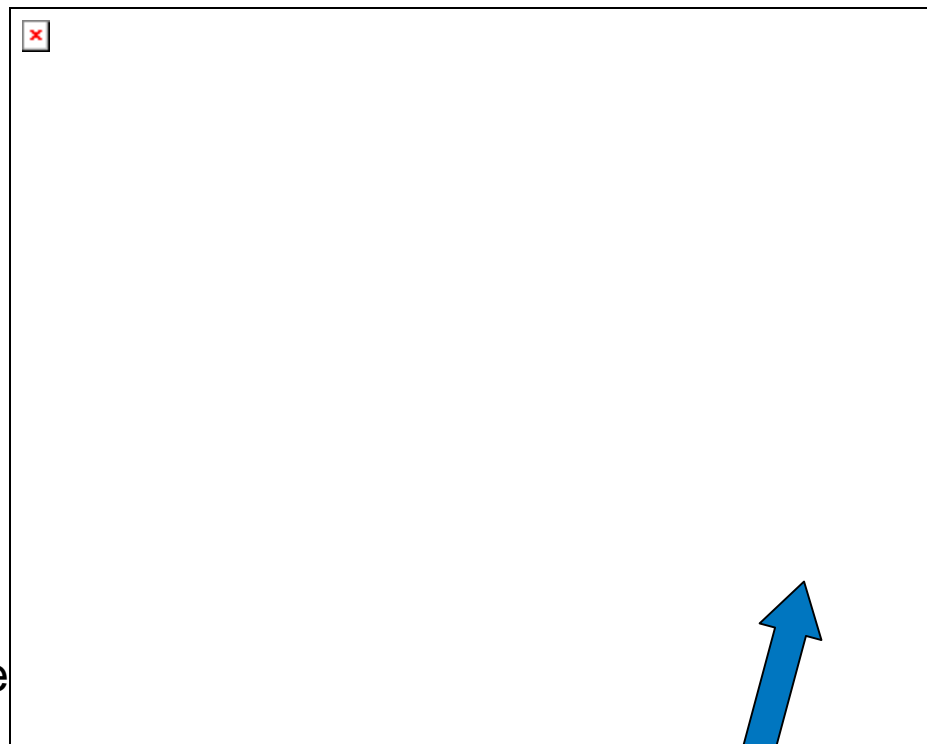
- The approach of performing SPME-DESI-MS/MS demonstrated a unique approach for rapid analysis of biological samples.
- The results from SPME-DESI-MS/MS indicate that this methodology is suitable for direct screening of urine samples with minimal sample preparation.
- The SPME-DESI-MS/MS combination provides a suitable method for combination for screening as well as quantitation of opiates in urine.

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

Inert to sample matrix  
Solvent-stable coatings

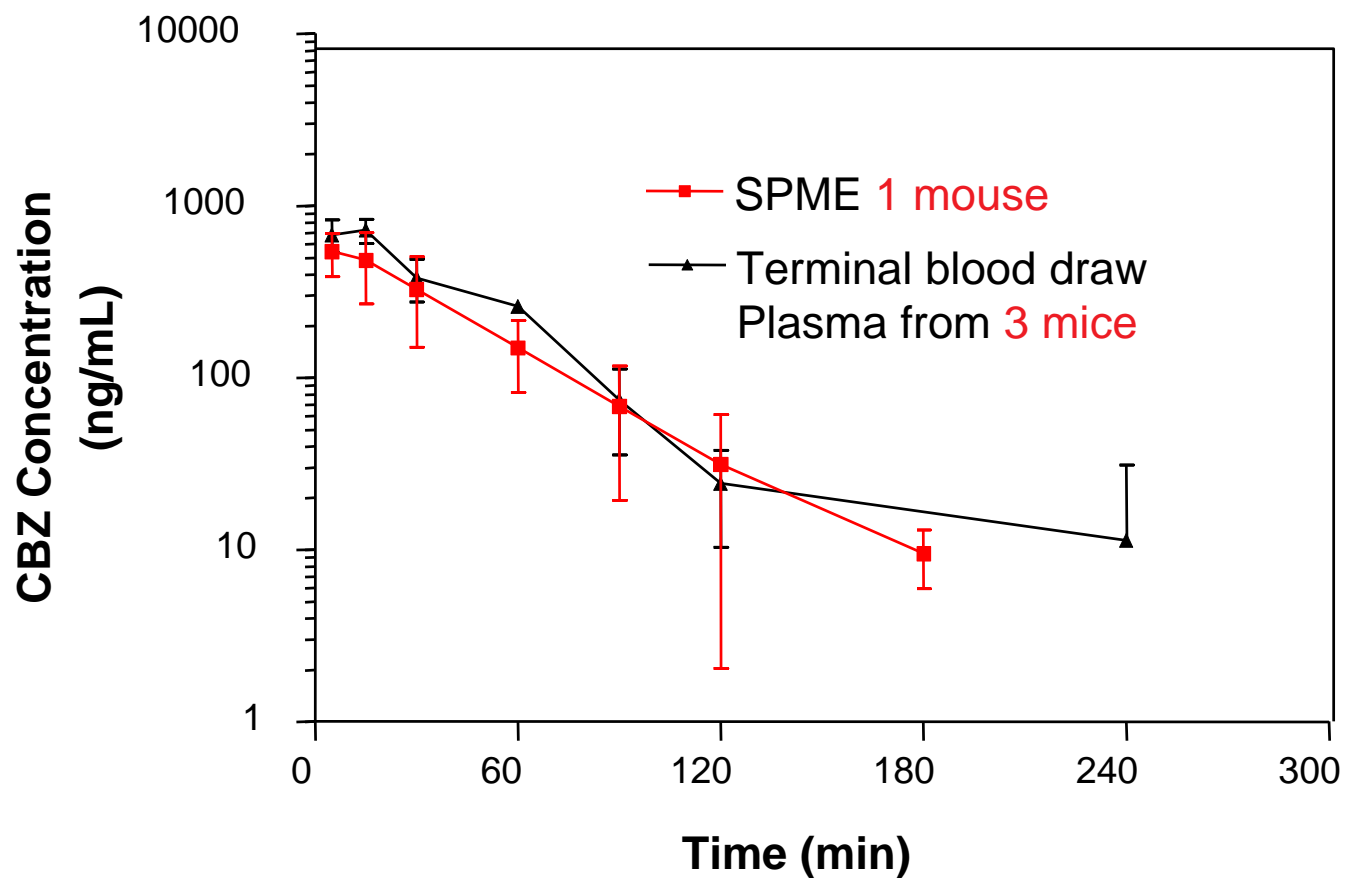
Ideal for:

- Difficult matrixes (plasma, tissue)
- Non-volatile analytes
- Living systems (e.g. animals, plants, cell culture)
- Multiple data points per sample
- Reduces lab animal sacrifice



For laboratory use only

# Comparison of SPME *in-vivo* PK Study of Carbamazepine from Mice Whole Blood to Extracts of Plasma Removed from Mice



Slide Courtesy of Ines de Lannoy, NoAb BioDiscoveries

# Summary

Classically GC, GC/MS

New developments in SPME coatings and devices coupled with the sensitivity of MS promises expanded utility

- Biofluid compatible proof-of-concept example
- in-vivo example

Imagine new specific chemistries.....simultaneous PL removal?

Imagine new uses – formats similar to DBS?

Challenge – what can we devise for your work?

# Acknowledgements

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