SPME-LC Fibers for a Variety of Applications

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Abstract

An SPME fiber has been developed that is specifically designed for solvent desorption. The fiber coating contains bonded HPLC particles embedded in a proprietary binder that has biocompatible properties. This presentation highlights the extraction of drugs such as carbamazepine and propranolol from biological fluids at therapeutic levels. Low cost devices have been developed that will allow single-use extractions for both *in-vivo* and *in-vitro* applications.

Introduction

All of the commercially available SPME fibers contain coatings primarily designed for thermal desorption and GC analysis. A new line of SPME fibers has been developed specifically for solvent desorption with biocompatible properties. Biocompatible fibers reduce surface adhesion of proteins and other large macromolecules while allowing the extraction of small analytes. Unlike the current multi-use fiber assemblies, these fibers are contained in devices that will make single-use extractions cost effective. This presentation highlights important parameters in the development of the coated fibers and devices. A variety of applications showing the extraction and analysis of small molecules from biological fluids will be presented.

Goals of Development of SPME Fibers for Solvent Desorption

1. Fiber coating must be durable and reproducible
2. Fiber coating must not swell in water or organic solvents
3. Must be able to coat HPLC particles on fiber
4. Binder should not affect uptake of analytes
5. Binder should be biocompatible
    a. Resists large macromolecules
    b. Can be used with *in-vivo* type experiments without harming organism
6. Device needs to be low cost for single use analysis

Experimental and Results

Fiber Coating - Process and Properties

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

Findings

- Binder is inert and does not swell in water or organic solvents
- Binder does not impede extraction of small molecules
- 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

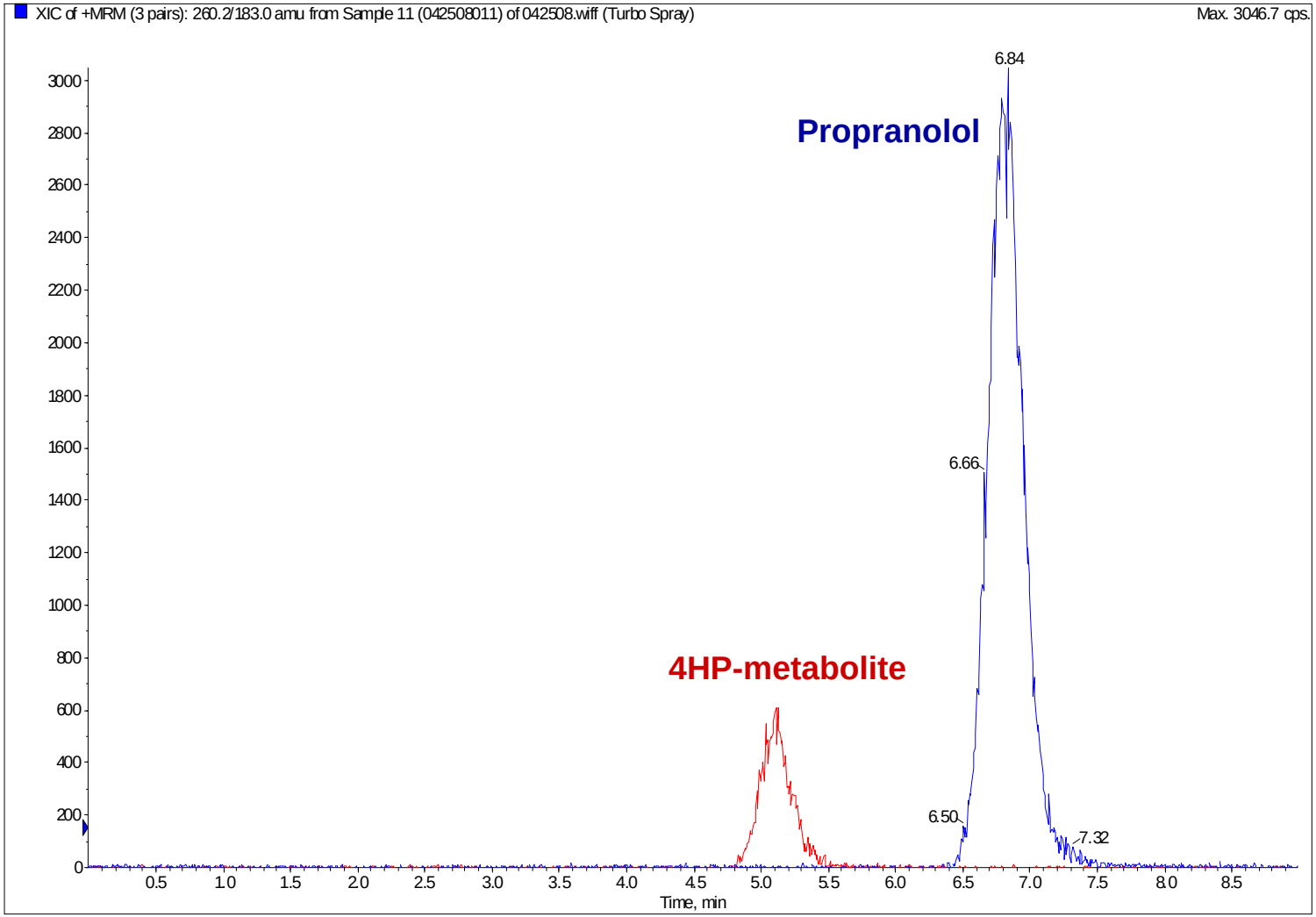
If swelling occurs, the coating can be more easily damaged and removed from the fiber core, especially when a fiber is retracted into a needle.
The results show that SPME fibers developed with GC type phases are subject to swelling in some solvents, but the newly developed fibers for solvent desorption do not swell in most commonly used extraction solvents.

Extraction of Propranolol and 4-Hydroxypropranolol (4-HP) Metabolite

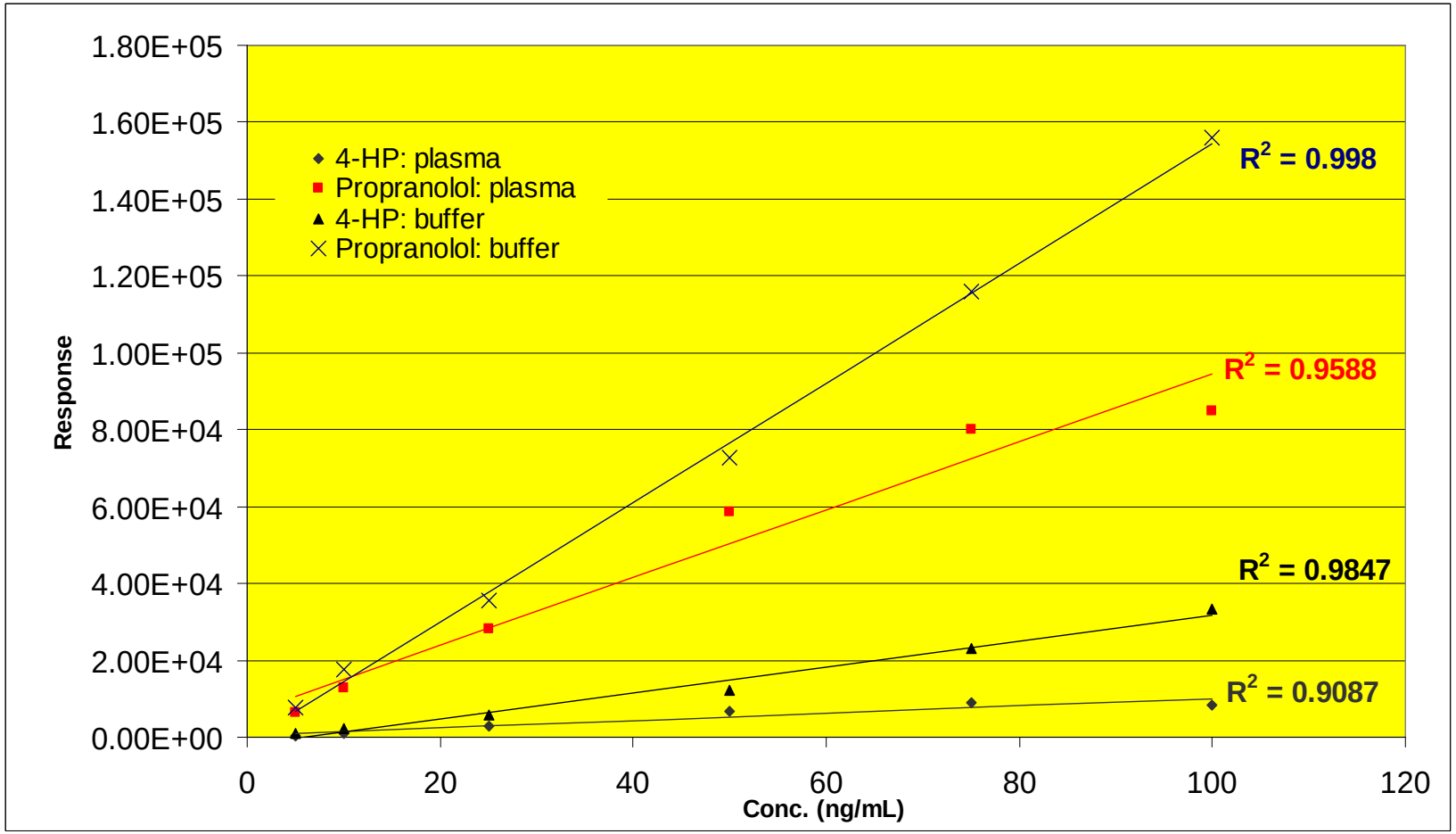
Procedure for Extraction and Analysis

Fiber Type	RPA 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, followed by 15 min. in water
Extraction	10 min., static
Desorption	60 min. in 100 µL 13 mM NH_4OAc in 90:10 ACN:H_2O
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

HILIC Mode Separation of Propranolol and 4-HP



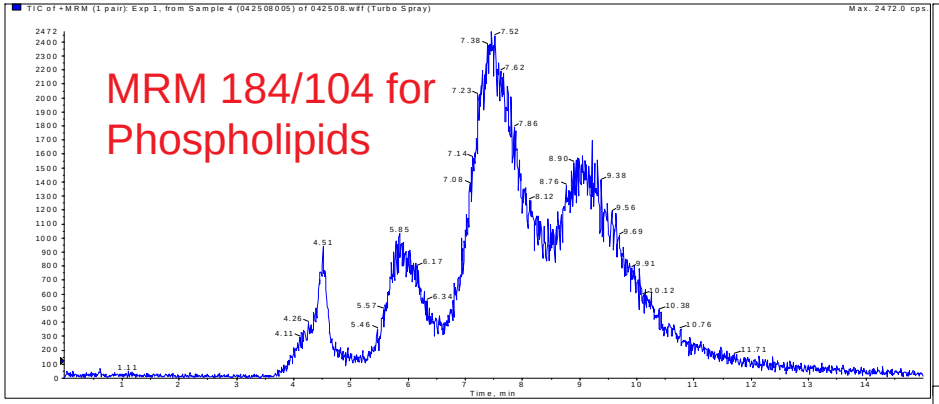
Linearity of Extractions of Propranolol and 4-HP from 100 µL Samples

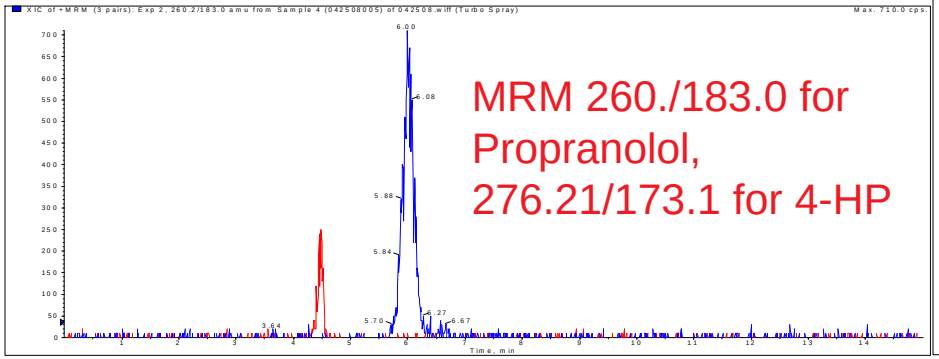


The results show that the analytes can be extracted out of a small volume of either buffer or plasma with good linearity at low concentration levels. The more polar metabolite is not extracted as efficiently as the parent drug. Also, the recovery of both analytes is less out of plasma compared to buffer. This is due to binding of the drugs to proteins in the plasma. It was shown that it can take multiple hours for the binding equilibrium between the drugs and protein to be met. If the extraction occurs immediately after spiking the drugs into the plasma, the difference in recovery between buffer and plasma is much smaller. Better linearity is obtained if full protein binding equilibrium is obtained. An advantage of SPME is that it only extracts free (unbound) drug that is therapeutically active.

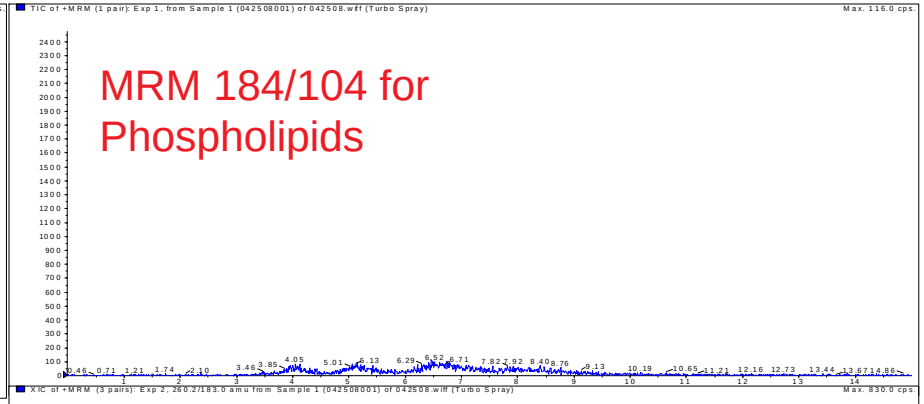
LC-MS Analysis of Drugs in Plasma: Comparison of SPME Extraction to Direct Injection on the Matrix Background and Detection of the Drugs

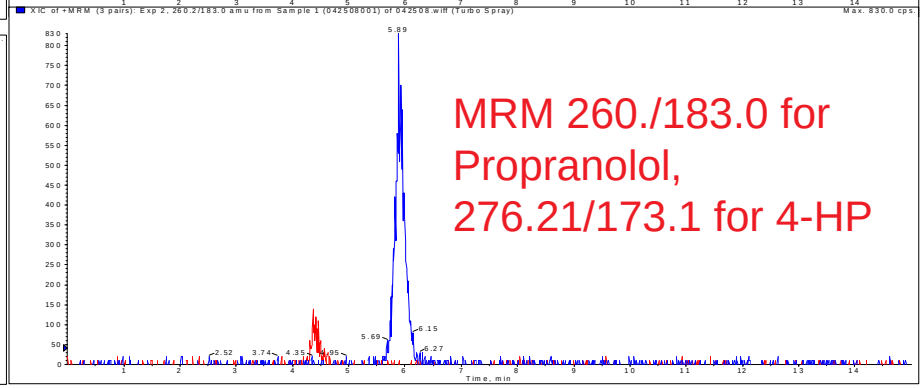
Direct Injection after protein precipitation





SPME Extraction





Phospholipids (PL) are large molecules that can interfere with the analysis of drugs by supressing ions, especially if the PL elute with the analytes of interest. It is common to analyze drugs in plasma by precipitating proteins followed by direct injection of the supernatant. Often not all of the PL precipitate when acetonitrile is added to the plasma which can result in supression specific for the drugs detected by the LC-MS system.

The above figures compare the analysis of the drugs and PL by precipitation of proteins in plasma followed by direct injection, to extraction of untreated plasma using SPME followed by desorption and analysis. The ion chromatograms are at the same intensity levels. The results show that SPME does not extract PL due to the biocompatible polymer, but the drugs of interest are extracted at similar levels to direct injection that contained a high concentration of PL.

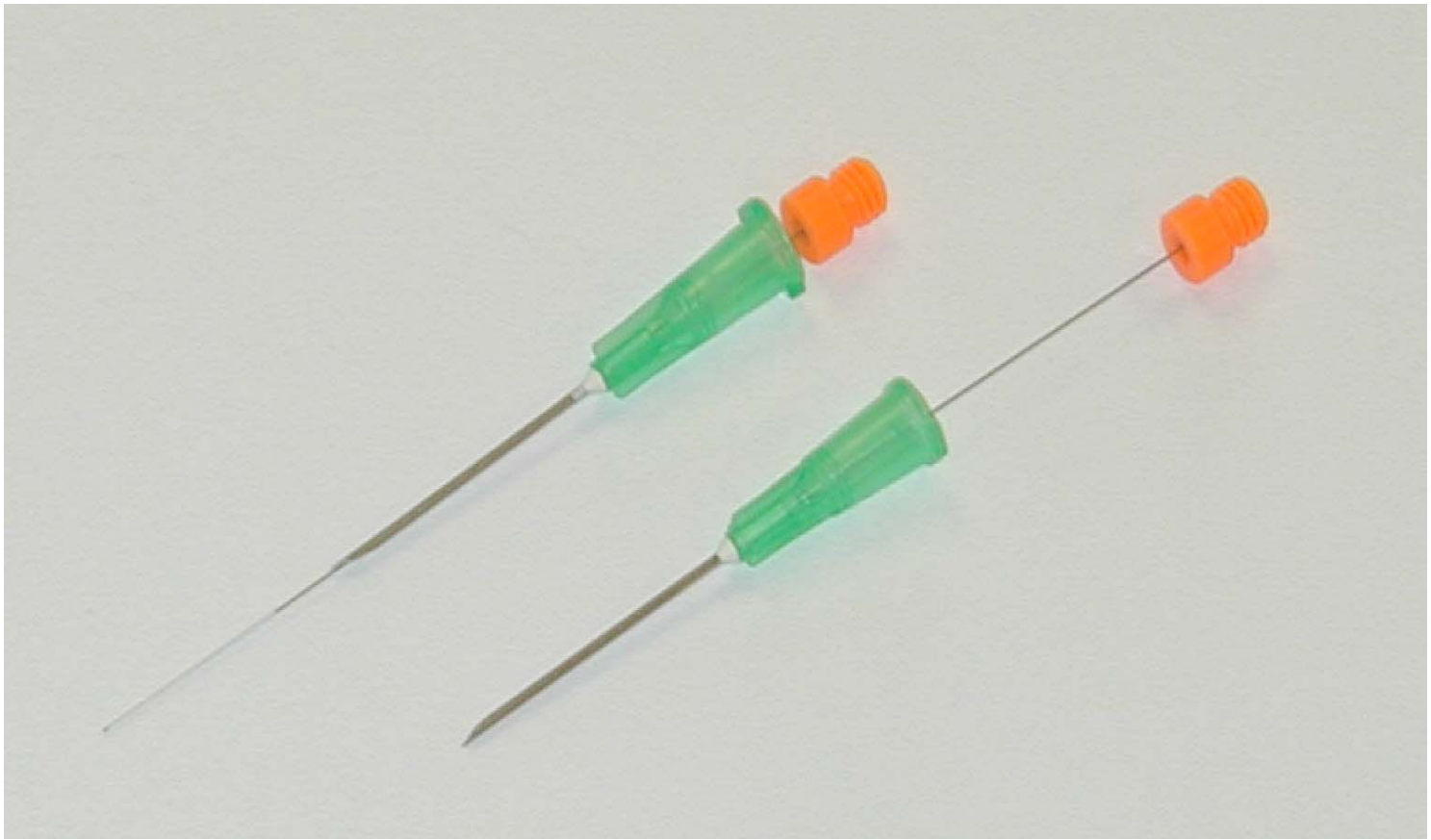
Fiber Pipette Design and Use

A low cost device has been designed that contains a coated SPME fiber in a disposable pipette tip. The tip can be inserted into low volume vials for extraction and desorbed in 50 µL of solvent contained in a 100 µL conical vial. Multiple samples can be extracted at one time and the desorption can be accomplished in one step with multiple vials and fibers. The vials containing the desorbed analytes can be placed in an autosampler for analysis. It is possible to put tips in a plate format to simplify the extraction process.

Reproducibility

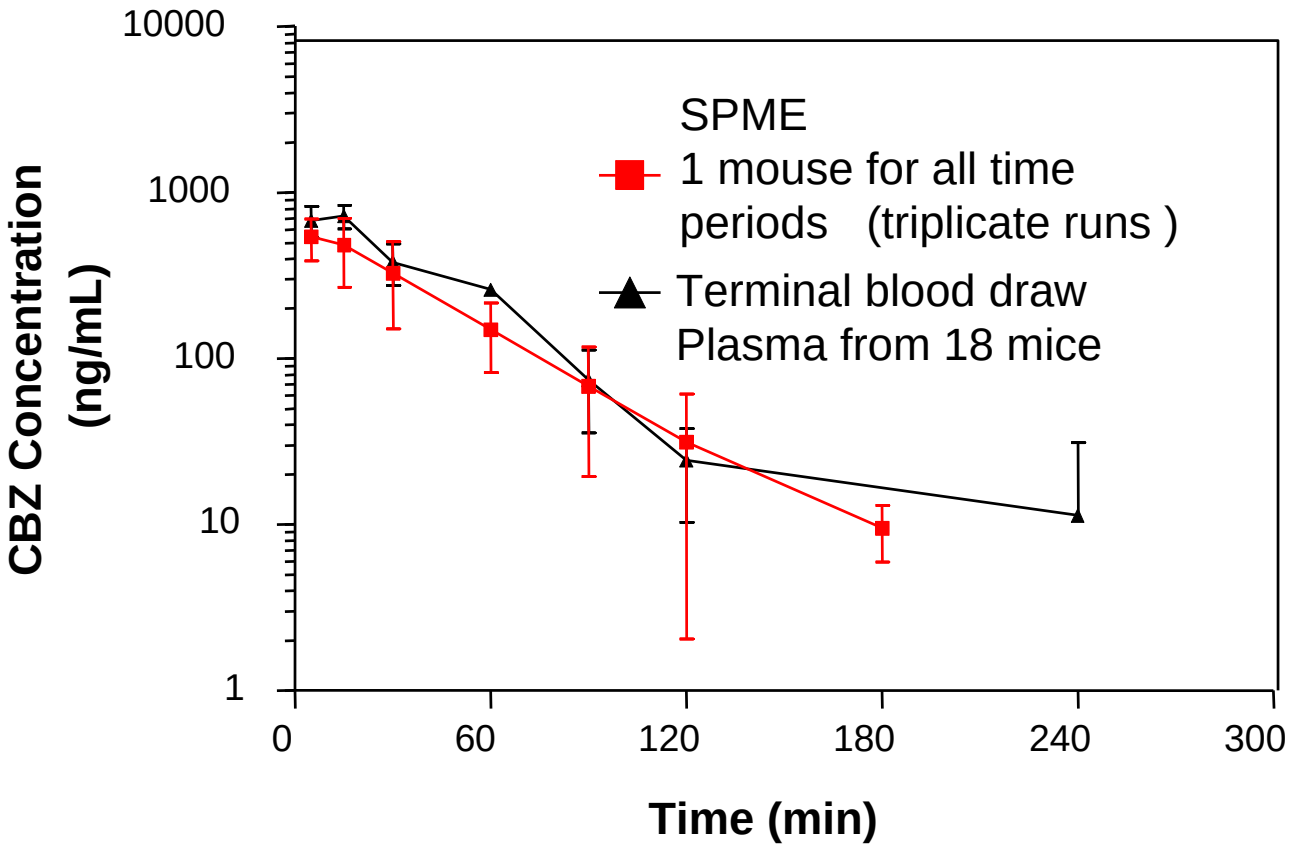
Reproducibility of the area response of drugs extracted with 10 fiber tips per lot, typically ranges between 3% and 9% relative standard deviation. Various drugs used to evaluate the tips include cocaine, oxycodone, propranolol and benzodiazepines along with their common metabolites. The variation was calculated without correction with an internal standard.

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



Probes have been designed so that blood could be sampled *in-vivo* from animals with the use of a shunt device. Also, probes could be directly inserted into plant and animal tissues for direct analysis of small molecules.

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.

The above slide shows the PK of CBZ in mice. A 2 mg/Kg dose of CBZ was given to mice and the level of the drug in the blood stream is monitored over time. The traditional way of monitoring the drug is to remove blood at various time intervals usually 6-8 times over a 24 hour period followed by extraction using SPE. Because mice have a very limited volume of blood, the doped mouse is sacrificed between 2 and 3 extractions. In this study triplicate samples are obtained at each time point; therefore, 18 mice were sacrificed to obtain plasma. This is costly and the data are slightly skewed because the metabolic rates can differ between mice.

The staff at NoAb BioDiscoveries developed a shunt device that passes the blood of the mouse through the shunt containing a port that allows the SPME fiber to be inserted for a 2 min. extraction of the drugs. Extraction of drugs with the fibers can be done multiple times without harming the mouse. For the SPME portion of this study 3 mice were used, with each mouse surviving the entire timed study.

Conclusions

- SPME fiber coatings have been specifically designed for HPLC use and bio-applications
- Fiber does not swell in water and/or solvents
- Fiber coating is biocompatible
- Fiber coating is durable and reproducible
- Fiber can be inserted into a pipette tip for easy handling and automation.
- Fiber probe is suitable for *in-vivo* and *in-vitro* applications
- Different coatings are being evaluated

Acknowledgements

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