K47 Direct Tissue Sampling of Diazepam and Amitriptyline Using Mixed-Mode Solid-Phase Microextraction (SPME) Fibers: A Feasibility Study

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After attending this presentation, attendees will understand the use of mixed-mode SPME fibers as direct tissue sampling tools to determine tissue concentrations of neutral and cationic compounds.

This presentation will impact the forensic science community by providing data on direct tissue sampling of diazepam and amitriptyline using mixed-mode SPME fibers.

Recent work with SPME fibers *in vivo* has shown that this technique is easily applied directly in semi-solid tissues; however, at this time, data on tissue sampling are still very limited, and adequate models to study sorption from tissue are lacking. Furthermore, quantification of actual tissue concentrations remains a challenge in the application of SPME in tissue.

The goal of this research is to evaluate the applicability of the C18/Strong Cation Exchange (SCX) -coated SPME fiber as a direct tissue sampling tool. The C18/SCX (mixed-mode) fiber coating consists of hydrophobic C18 chains and SCX groups, made up of propylsulfonic acid.

Diazepam and amitriptyline were used as test compounds to demonstrate that this fiber can efficiently extract both neutral and cationic compounds. Diazepam is neutral, and its behavior is predictable based on the octanol-water partition coefficient (K_{ow}). Amitriptyline is >99% positively charged at pH 7.4 and is likely to behave differently in both agarose gel and tissue compared to neutral compounds. Agarose gel was used as a tissue surrogate to mimic changes in matrix tortuosity as expected in tissue. Pork muscle was used as tissue source and was loaded with the analytes of interest using 24-hour incubation in spiked Phosphate-Buffered Saline (PBS).

Linear sampling isotherms were observed for agarose gel. The results with tissue were more complex as the cubes of muscle meat were difficult to equilibrate to a homogeneous loading concentration in the applied test systems. This influenced the sampling kinetics and extraction linearity with unknown uncertainty. Still, the C18/SCX fiber extracted both diazepam and amitriptyline from the muscle tissue and, when diazepam concentrations were higher in tissue, similar high levels were determined via microextraction.

Sorption affinity of both diazepam and amitriptyline is decreased (±1 log unit) when sampling from agarose gel compared to PBS due to the presence of different binding groups in agarose. When comparing sorption affinities between agarose gel and tissue, sorption affinity from tissue is ±1 log unit lower for diazepam, while this is ±2 log units for amitriptyline. This indicates that tissue contains even more binding sites for these compounds compared to agarose gel. Interestingly, for both compounds, equilibration in tissue occurred faster than equilibration in agarose gel or PBS, most likely caused by direct fiber contact or through facilitated transport.

The proposed SPME method yielded detectable fiber concentrations after direct sampling in agarose gel and loaded tissue, including short sampling times and different loading concentrations in tissue. Although more research

is needed to obtain good quantitative results, these results illustrate that the C18/SCX fiber is a sensitive tool to determine tissue concentrations of neutral and cationic compounds.

In future research, such quantitative measurements must be pursued to apply the current SPME methodology in forensic toxicology. An example of this can be studying postmortem drug redistribution. SPME fibers can be placed directly *in situ* in tissue or blood without removal of these matrices. As shown here, only a short time interval is needed to obtain detectable fiber concentrations. Furthermore, SPME does not disturb the existing equilibrium in the body as only very small amounts are extracted. This would allow for repeated sampling in the same system for a prolonged period. This could eliminate the current difficulties in studying postmortem redistribution as a kinetic study can be executed in a single postmortem case.

Solid-Phase Microextraction, Tissue Sampling, Agarose Gel