

GETTING STARTED WITH SPME

SYSTEM STARTUP ESSENTIALS

The Combi PAL is a versatile and simple system for automated SPME analysis. To take full advantage of the system there are several points to consider before beginning automated sample extraction and loading.

1. This guide assumes familiarity with the fundamentals of SPME method development. Beginning users are referred to “Solid Phase Microextraction: Theory and Practice” by Janusz Pawliszyn or check out his web Site at <http://www.science.uwaterloo.ca/chemistry/pawliszyn>.
2. Septum coring is a common problem to most inlets used with the blunt-tipped SPME needles. There are several solutions for this problem. Use either a pre-bored septa or get a Merlin Microseal. The pre-bored septum is inexpensive and tends to have less likely any sealing problems, if frequently changed. Both 23ga and 24ga SPME needles are available from Supelco. We recommend the 23ga needle configuration for durability.
3. Choice of inlet liner can have a significant effect on the quality of the chromatography. Desorption is most efficient when narrow bore ($\leq 1\text{mm}$) liners optimized for SPME are used. These liners provide high carrier gas linear velocity across the fiber, giving most efficient analyte desorption and the best peak shapes. The 0.8mm liner should be used for best performance with 24ga fibers. *Supelco part #: 204780-x (x = number of liners)*
4. SPME fibers are available in a variety of phases and are configured for either manual or autosampler use. If you are currently using manual SPME and have a supply of manual fibers, these can be converted for use in the Combi PAL by removing the spring from the fiber by unwinding it. Typical lifetime of a fiber is at least 100 samples.

Sample vials and septa should be chosen to meet the demands of each application. Because of SPME’s high sensitivity, septum bleed in the vials during sampling may result in significant background during the SPDE run. It’s best not to use very hard or sticky septa, since the needle can’t penetrate easily through it. Moreover sticky septa won’t release the needle while the system tries to move to the next object. We recommend using screw-top vials from La-Pha-Pack. They are the only recommended vials from CTC Analytics and are available from CHROMSYS. For SPDE, we recommend using the viton septa for its excellent background performance. The part number is: 18 03 1424 – 10 or 20mL, depending on vial size.

SPME Fiber Selection

Supelco sells a variety of SPME fibers and has published a series of application notes describing a wide range of SPME applications. These and other literature publications may be helpful in selecting a few fiber types to try for new applications. The table below contains a brief summary of typical applications for different fiber types.

Fiber Type	Application
Carboxen/polydimethylsiloxane	Low MW volatiles (highest capacity)
Polydimethylsiloxane	General volatiles
Polydimethylsiloxane/divinylbenzene	Amines/nitroaromatics
Polyacrylate	Polar semivolatiles (selects against nonpolars)
Carbowax/divinylbenzene	Alcohols/polars
Divinylbenzene/carboxen	Broad range sampling (volatiles+semivolatiles)

- The standard fiber used for automated SPME is 1cm long. Default parameters programmed into the Combi PAL assure the entire fiber is exposed while sampling and during desorption in the inlet. Supelco also makes a 2cm fiber that can be used with the Combi PAL and may give higher sample capacity. Special precautions must be taken to use needle penetration and fiber penetration depths that assure the entire fiber is exposed during sampling and desorption. Please be aware, that 2cm fibers are more fragile. Consider purchasing a Single Magnet Mixer, when using the 2cm fibers.

ADDITIONAL HARDWARE

The **Fiber Bakeout Station** can be used to pre-condition fibers outside of the GC, preventing any fiber contamination in your column or inlet. The item is fully integrated within the firmware. It will allow you to condition your fiber after desorption in the inlet. This is especially important if your inlet desorption temperature is too low to sufficiently clean the fiber for the next run.

The **Single Magnet Mixer (SMM)** is a heated (optional Peltier cooling available) stirring device. The stirring is performed by a magnet, which is placed inside the vial. This object is useful if you intend to do more liquid SPME than headspace SPME. The SMM is gentle to the fiber while it is immersed in the liquid. This is especially true of 2cm fibers. The higher efficiency of stirring as opposed to shaking, generates higher responses for semi-volatiles.

SPME Fiber Preconditioning

New fibers must be preconditioned before use to eliminate background interference and prepare the fiber for reproducible sampling. The maximum temperature the fiber can tolerate, and the recommended conditioning protocol (temperature and time) will depend on the polymer phase on the fiber. Supelco provides technical data sheets with their SPME fibers that list all the information necessary to select appropriate conditions.

After installing a new SPME fiber in the Combi PAL injection unit, the most convenient means of preconditioning the fiber is to set up methods on the Combi PAL, that automatically bake the fiber in the Fiber Bakeout station or the GC inlet under the appropriate conditions. If the inlet is used, please set it in split mode with a high split flow, this efficiently cleans the fiber and prevents most contaminants from being introduced onto the column. Method "FiberCon" for preconditioning a new fiber is described in the Sample SPME Methods section below.

Once a new fiber has been preconditioned and the background in blanks is acceptable, it is usually not necessary to extensively recondition the fiber between samples if fiber conditioning is built into the desorption protocol. Typically, fiber desorption is done in the splitless mode into a hot inlet. If the fiber is allowed to remain in the inlet for about 5 minutes after the split vent is opened and a high split flow is used, the fiber will be cleaned of any residual sample and will be ready for the next sampling. Method "SPMEBAKE" for reconditioning a fiber is described in the Sample SPME Methods section below.

SETTING UP THE INLET FOR SPME

If you are using a standard hot split/splitless inlet, purchase pre-bored inlet septa. Otherwise watch for accumulation of septum cores in the inlet liner, which will give increased siloxane background and can lead to fiber breakage if the liner becomes blocked.

SAMPLE SPME METHODS

FiberCon - Used for initial conditioning of a new SPME fiber

Combi PAL

Pre-incubation time	0 min
Incubation Temp	Off
Extract Time	10 sec
Desorb Time	Refer to Supelco Technical sheet for recommendations for each individual fiber type. Typically, 1-4 hr.

Standby Temperature

Fiber Bakeout Station 250°C (refer to Supelco Technical sheet for conditioning temperature)

Or in the Inlet

Initial Temperature (250°C) Use hot inlet for SPME desorption

GC Runtime GC oven program + oven cool down cycle + GC equilibrium time

Mode Split

Split Ratio 30:1 or greater

Split Flow 30ml/min typical

SPMEBAKE - Used to recondition a fiber prior to beginning a new series of analysesCombi PAL

Pre-incubation time 0 min

Incubation Temp Off

Extract Time 10 sec

Desorb Time 10 min

Standby Temperature

Fiber Bakeout Station 250°C (refer to Supelco Technical sheet for conditioning temperature)

Or Inlet

Temperature (250°C) Use hot inlet for SPME desorption

GC Runtime GC oven program + oven cool down cycle + GC equilibrium time

Mode Split

Split Ratio 30:1 or greater

Split Flow 30ml/min typical

Test SPME - Example of a typical set of parameters for SPME analysisCombi PAL

Pre-incubation time 5-20 min

Incubation Temp 35°C-70°C (Application dependant)

Extract Time 3-20 min (Application dependent)

Desorb Time 5 min

Fiber Bakeout 10 min (after injection)

Inlet

Temperature (180-250°C) Use hot inlet for SPME desorption

GC Runtime GC oven program + oven cool down cycle + GC equilibrium time

Inlet Parameters

Mode: Splitless
Purge Flow to Split Vent: 20ml/min or greater
Purge Vent Time: 0.9 min

SPME Setup for the Combi PAL

Refer to the SPME manual supplement provided with your SPME kit for details on setting up the Combi PAL for SPME operation. Below are a few highlights and clarifications of procedures in the SPME manual.

Note: SPME fibers can easily be damaged while mounting and dismounting the fiber holder adapter in the injection unit. We recommend mounting the holder with no fiber installed to practice aligning the locating pins in the injection unit.

Install the desired SPME fiber in the fiber holder, and be sure the fiber is fully retracted into the protective needle. **Failure to assure the fiber is fully retracted while mounting the fiber holder in the injection unit can result in irreparable damage to the fiber!** To install the SPME fiber holder in the Combi PAL injection unit, the Combi PAL must first be moved to the Change Syringe position using the keypad. This lowers the injection unit and allows access to the plunger holder. Remove the installed syringe holder and carefully install the SPME fiber holder by first inserting the SPME needle through both upper and lower needle guides. After the needle passes smoothly through both needle guides, raise the SPME holder adapter until the lower locating pins snap into their corresponding holes in the injection unit. The top of the SPME holder then will snap magnetically into the injection unit. Secure the SPME plunger with the gold plunger holder. The plunger may have to be lowered to attach the plunger holder; as long as the needle is properly aligned in the needle guides the fiber will not be damaged.

When preparing to run SPME methods with the Combi PAL there are a few extra steps necessary to teach to the Combi PAL. **Caution! Use care in teaching or checking positions of objects in the Combi PAL if the SPME fiber is mounted in the injection head. If incorrect coordinates are set and the injection unit drives the SPME fiber downward into an object the fiber can be irreparably damaged.** If many object positions must be taught (as when setting up a new system) it is safest to remove the SPME holder adapter first.

The FiberExp position is used by the Combi PAL to lock the needle guide in the “up” position to allow sample agitation while the fiber is inserted in the vial. This position must be defined properly for the SPME cycles to run successfully.

The Combi PAL is preset with default parameters for needle penetration in the injection port (40mm) and vial penetration (22mm). The injector needle penetration depth has been optimized for use with

liquid or SPME injection on the Split/Splitless inlet. The vial penetration default is appropriate for headspace or liquid SPME extraction depending on vial size (10ml or 20ml) and sample volume.

Please use soft septa and large hole size caps to ensure a larger target area for the fiber. Standard 2mm holes will do for most injections, but after a few injections the needle will start to bend in any direction. This usually happens close to the end of a fibre's lifetime.

Within the SPME method is a parameter called "injector penetration" that determines the position of the bottom of the SPME fiber in the inlet. For standard 1cm fibers this value should be 54mm.

If the agitator temperature and "On Time" are shut off, the Combi PAL will not transport sample vials to the agitator. Instead, it samples directly from the vials in the trays.