ExtraTECH
- ANALYTICAL SOLUTIONS -

Vac-SPME Fiber and Arrow Instructions Manual

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Table of Contents

Vacuum sampling with SPME Fiber and Arrow

Solid phase microextraction (SPME) is a solventless sample preparation technique, commonly used to extract organic compounds from solid, liquid, and vapor matrices. In the headspace sampling mode, analytes are transferred from the sample matrix to the headspace above it and selectively absorbed/adsorbed to the SPME fiber. Over time, the analyte amounts in the SPME extracting phase reach equilibrium level with their surroundings. This equilibrium state corresponds to the maximum solute amounts that can be extracted under a given set of sampling conditions. Volatile compounds reach equilibrium typically within few minutes, while analytes with a low affinity for the headspace (e.g., semi-volatiles) require longer sampling times that may exceed 60 min when sampling at room temperature. Heat and agitation are usually applied to enhance headspace concentrations and speed up the time necessary for reaching equilibrium. Nonetheless, heating may degrade the sample, enhance matrix effects, or decrease the partitioning constants between the fibre and headspace.

SPME sampling under vacuum (Vac-SPME) has been found to accelerate the extraction kinetics of analytes exhibiting long equilibration times via regular HS-SPME at atmospheric pressure. At the same time, the extraction of volatiles that reach equilibrium fast is not affected. Another important feature of Vac-HS-SPME is the mild sampling temperatures applied that allow for preserving the composition of the sample and prevent thermal decomposition of labile analytes. The vacuum approach is in fact so powerful that it can even be applied for sampling volatiles at a sub-ambient temperature (i.e., 5°C). This is crucial when sampling in close-to-real-world scenarios, and for preventing the formation of by-products due to sample heating at elevated temperatures.

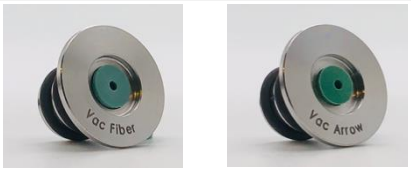
The positive effect of vacuum was initially demonstrated for headspace solid-phase microextraction (**SPME Fiber**) and has since been extended to high-capacity sorbents, such as **SPME Arrow**. Today, vacuum sampling represents an additional experimental parameter to consider for achieving faster extraction under mild conditions whilst using different headspace microextraction technologies.

1. Product information

1.1 Vac-SPME Fiber and Arrow closures

Two types of Vac-SPME caps are available, compatible with either SPME fiber or SPME Arrow technologies. The key difference between the two lies in the septum they can accommodate. To distinguish between the two, “Vac Fiber” is engraved on the closures designed for SPME Fiber, and "Vac Arrow" on those for SPME Arrow (Figure 1).

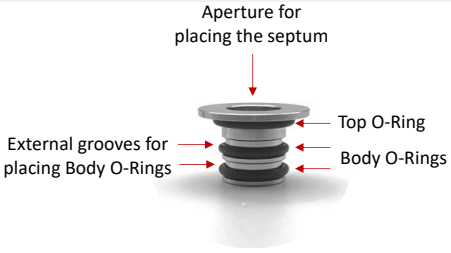
Figure 1: Vac-SPME Fiber and Arrow closures



Vacuum assisted caps are made of **a special type of stainless steel with magnetic properties. This allows direct handling by the robotic arm of an appropriate autosampler and eliminates the need for additional magnetic caps**.

Each closure has an aperture on the top to accommodate a septum through which air evacuation and SPME operations take place. Additionally, the closure is designed with two external grooves capable of accommodating up to two Body O-rings (Figure 2).

Figure 2: Side-view of the closure



To fit the Vac SPME closure into the sample vial neck, **twist and push the closure at the same time**, until it is fully inserted. Twisting the closure whilst pushing is necessary to fit the closure and ensure that O-rings are positioned correctly.

The metallic body of the closure exhibits **good chemical resistance to a wide range of chemicals and is corrosion-resistant** to water, water vapor, weak organic acids, dilute solutions of nitrates, carbonates and other salts. However, the closures should be handled away from **strong oxidizers, highly acidic or basic solutions, and prolonged contact with high ionic strength solutions**.

1.2 Vials

Vac-SPME caps provide a gastight seal when utilized with commercial **20 mL, 20mm crimp top headspace vials** (Figure 3). When employing an autosampler, ensure that the vial used is approved and recommended for use with the autosampler. The inner neck diameter of the 20 mm crimp vials may vary among different suppliers and the presence of O-rings allows for accommodating such small variations. In cases where closures do not fit into the vial, packs with a different product number or from another supplier should be used.

The 20 mL, 20 mm crimp top headspace vials come with a long or short neck (Figure 3). **We recommend the use of long neck vials as they offer an increased contact area with the closure compared to the short neck ones. For these vials one or two Body O-rings can be placed on each external groove. However, when using short neck vials always place two Body O-Rings on each groove to allow multiple sealing areas with the neck** (Figure 3).

Figure 3: Body O-ring configurations for short and long neck vials

1.3 Septa

Two different types of septa are used with the Vac-SPME Fiber and Arrow closures. **To fit each septum in the opening of the closure, gently twist and push until it is fully inserted**. Septa that are not fully inserted and exceed the upper surface of the closure may obstruct automated operations. To remove and replace the septum, use tweezers, a pin or a septum puller. Gloves must be worn to prevent contamination when fitting septa into closures.

1.3.1 Septa for Vac-SPME Fiber closures

The aperture on top of each **Vac-SPME Fiber** closure can tightly accommodate a Thermogreen® LB-2 plug septum (Figure 4). The septum is manufactured from high quality material, tested for bleed and contamination, and is already conditioned. According to the manufacturer, **the plug septa can be pierced up to 100 times and still maintain the gas-tight seal**.

Figure 4: Plug septum for Vac-SPME Fiber

1.3.2 Septa for Vac-SPME Arrow closures

The aperture on top of each **Vac-SPME Arrow** closure can tightly accommodate a Thermogreen® LB-1 half-hole septum (Figure 5). **Prior the first use, this type of septa must be conditioned for 16 h at 150 °C to avoid outgassing of siloxanes and contamination**. After conditioning they can be stored in clean containers until use. **The cylindrical septa can be pierced up to 50 to 100 times without irreversible damage**.

Figure 5: Cylindrical septum for Vac-SPME Arrow

1.4. O-Rings

Two types of Viton® O-rings are used. It is important to note the differing dimensions of the two O-ring types and where they are used:

- **Top O-Ring (Part number: C-VAC-O-TOP)** is the larger and thicker one that fits on the top part of the closure, creating a seal between the closure and the flat top of the vial mouth. One Top O-Ring is placed on each Vac closure.
- **Body O-Ring (Part number: C-VAC-O-BODY)** fits onto the two grooves of the main body of the closure creating a seal between the closure and the inner side of the vial neck. Each groove can accommodate up to two Body O-Rings. For long neck vials, one or two Body O-Rings can be placed on each groove. For short neck vials, two Body O-Rings on each groove are necessary to ensure gastight seal (Figure 3).

Viton® O-rings have a high-temperature resistance and can be placed in drying ovens. For water samples, the recommended max. temperature during Vac-SPME sampling is + 80°C. Both types of O-rings show no contamination and have good durability. **They are resistant to alcohols, aromatic hydrocarbons, and chlorinated hydrocarbons among others but are not compatible with ketones (e.g. acetone), low molecular acids, amines and alkalis, strong oxidizing agents, and strong acids/bases**.

2. Air evacuation of the sample container

2.1 Getting setup – manual and mechanical air evacuation

Vacuum-assisted methods preserve the simplicity of the standard methods, and the only extra step needed is the removal of air from the sample container before SPME.

2.1.1. Manual air evacuation

In principle, air evacuation of the sample container can be performed manually, by hand, using a gastight syringe. In this case, the air must be pulled several times to ensure low pressure conditions in the vial.

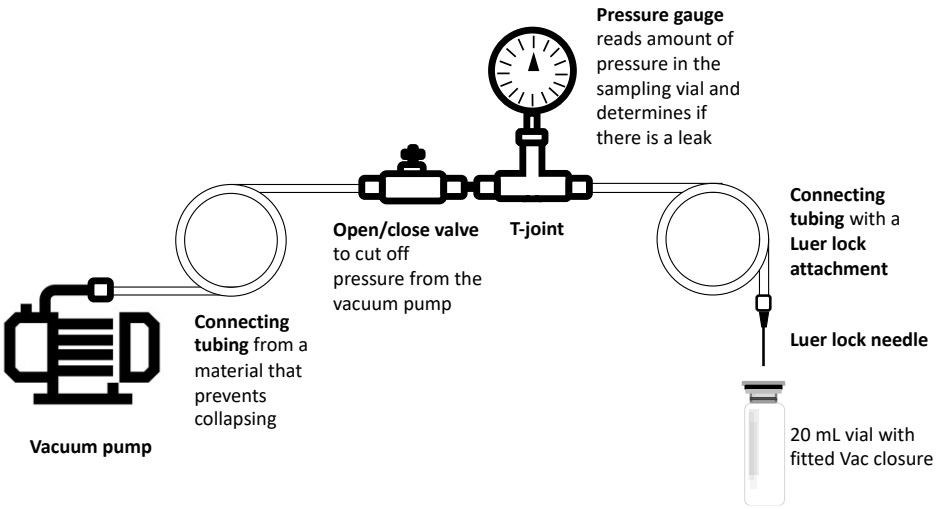
2.1.2 Mechanical air evacuation

The use of a vacuum pump is a more effective way to evacuate the air from the sample container and protects the septum from multiple punctures. **We recommend pumps having at least 7 mbar ultimate vacuum** such as MZ 2 NT (ultimate vacuum: 7 mbar), MZ 2C NT (ultimate vacuum: 7 mbar), MZ 2D NT (ultimate vacuum: 4 mbar) or MD 4C NT (ultimate vacuum: 1.5 mbar) from Vacuubrand (Wertheim, Germany), or MVP 10 basic vacuum pump (ultimate vacuum: 7 mbar) from IKA (Staufen, Germany). It is noted however, that the use of less powerful vacuum pumps, such as WP6111560 from MilliporeSigma (Burlington, MA), were reported as sufficient to effectively remove the air from the sample container. As a rule of thumb, **operational pressures must be below -750 mbar (approx. -22 inHg) to observe the positive effect of reduced pressure on SPME sampling.**

2.1.3 Pressure measurement and air evacuation

To develop a reproducible Vac-SPME method and detect leaks, a simple setup is recommended in Figure 6. In this setup the vacuum pump is connected in series to an open/close valve that cuts off pressure from the vacuum pump when desired, and a T-joint that is connected to the pressure gauge (digital or mechanical) and tubing. The other end of this tubing has a Luer lock attachment, which accommodates a Luer lock gas needle (e.g. replacement needles for Luer lock syringes or disposable medical syringes). Gas needles must be 23G or 22G. Smaller diameter needles are not recommended as they can be easily blocked by septum fragments.

Figure 6. Recommended setup for air-evacuation and pressure measurement



Steps to ensure enough vacuum has been pulled from the sampling vial are as follows:

1. Turn on the vacuum pump.
2. Insert the Luer lock needle into the septum of the closure for a predetermined period. In empty vials, the reading must be preferably below -800 mbar (approx. -24 inHg). In the presence of the sample, the reading must be preferably below -750 mbar (approx. -24 inHg).
3. Close the valve to verify that there are no leaks and confirm the vial pressure.

Stand-alone vacuum gauges can also be used to measure the pressure inside a vial. In this case, a Luer lock or Luer slip syringe needle is gas-tightly secured directly to the hose tail of a digital or mechanical vacuum gauge. In stand-alone vacuum gauges, the dead volume of the needle tubing and hub must be always minimized. To use a stand-alone vacuum gauge, insert the needle into the air evacuated vial with fitted closure and read the vial pressure. The use of a stand-alone vacuum gauge does not guarantee that low-pressure conditions will be maintained after measuring the pressure. For this reason, their use is mainly recommended at the end of analytical runs or for detecting leaks.

2.1.4 Determination of the minimum air evacuation time

Before starting the analysis, it is advised to determine the minimum time required for air evacuating the sampling vial. This time can vary depending on the vacuum pump, tubing, and sample vial volume. For every setup, the minimum air evacuation time must be determined at an initial stage, *i.e.,* during method development, and used thereafter. This will save time and will also minimize the aspiration of volatile analytes in case air removal takes place in the presence of the sample. Depending on the setup used, air evacuation times may vary from 30 s to 120 s.

3. The air evacuation step for liquid and solid samples

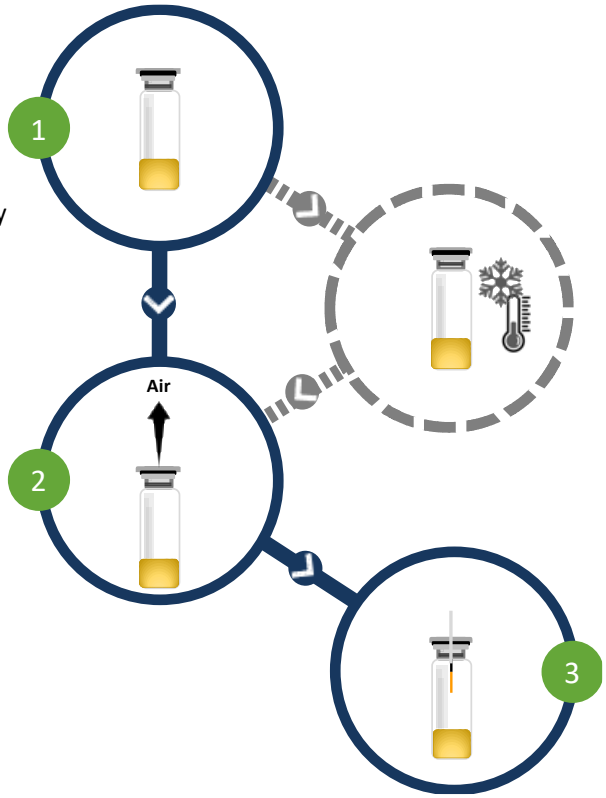
For liquid samples, air evacuation can proceed before or after placing the sample in the vial, whereas for solids, the only option is to place the solid sample in the vial before evacuation.

Air removal in the presence of the sample should not affect the extraction of semi-volatiles but can lead to aspiration of the more volatile analytes, particularly when long air-evacuation times are applied (e.g., above 90 s). **Freezing solid samples prior to air evacuation is one way to minimize volatile losses during aspiration**. At such low temperatures, headspace concentrations decrease, thereby minimizing the portion of volatile analytes that can be aspirated during air evacuation.

The steps for air-evacuating the sampling vial before or after sample introduction are as follows:

Air evacuation after placing the solid or liquid sample in the sample container

Step 1. Place the solid or liquid sample inside the vial. Fit the Vac closure equipped with a septum and O-rings. Freeze samples or proceed directly to Step 2.



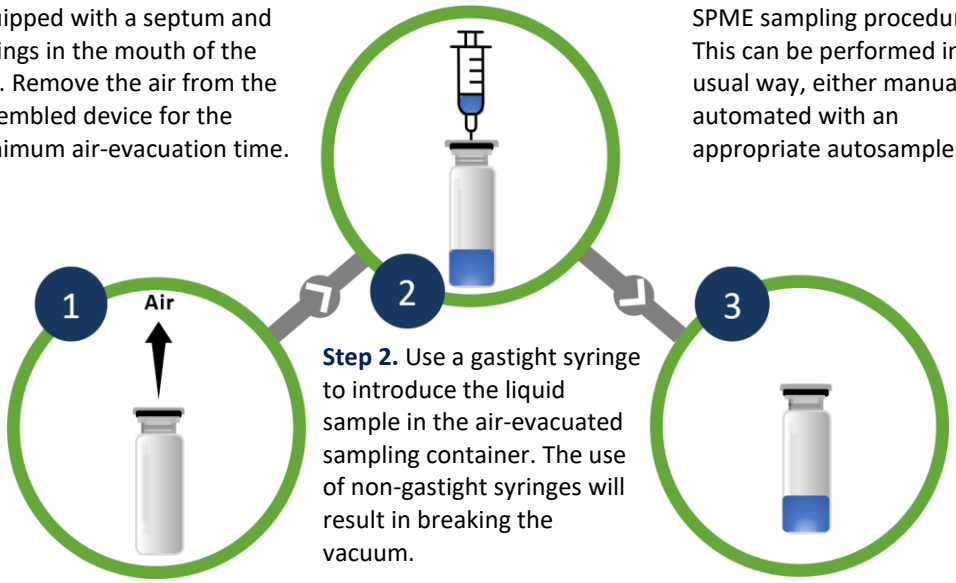
Optional Step. Place the sample in the freezer overnight or until frozen and proceed to Step 2.

Step 2. Remove the air from the assembled device for the minimum air-evacuation time.

Step 3. Proceed with the SPME sampling procedure. This can be performed in the usual way, either manual or automated with an appropriate autosampler.

Air evacuation before placing the liquid sample in the sample container

Step 1. Fit the Vac closure equipped with a septum and O-rings in the mouth of the vial. Remove the air from the assembled device for the minimum air-evacuation time.



Step 2. Use a gastight syringe to introduce the liquid sample in the air-evacuated sampling container. The use of non-gastight syringes will result in breaking the vacuum.

Step 3. Proceed with the SPME sampling procedure. This can be performed in the usual way, either manual or automated with an appropriate autosampler.

Upon completion of sample extraction, the pressure inside the vial can be equilibrated from under vacuum to atmospheric by piercing the septum with e.g., an open-end disposable medical syringe. The Vac SPME closure can then be removed, cleaned, and reused. Any deteriorated O-rings and septa can be replaced at this stage.

4. Handling, storage, and cleaning

- **This product is intended for use under vacuum pressure conditions and standard safety precautions must be taken**.
- The metallic closure has magnetic properties **allowing direct handling by the robotic arm of an appropriate autosampler without additional magnetic caps**. Caps and accessories should be stored in clean containers. Ensure gloves are worn to prevent contamination when handling the closures and accessories.
- The metallic body of the closure has a **good chemical resistance to a wide range of chemicals and solvents** and is corrosion resistant to water, water vapor, weak organic acids, dilute solutions of nitrates, carbonates and other salts. The closures must be handled away from **strong oxidizers, alkalis and highly acidic of basic solutions.** Extended contact with high ionic strength solutions must be avoided.
- The Viton O-rings are **resistant** to alcohols (e.g., ethanol, isopropanol), aromatic hydrocarbons and chlorinated hydrocarbons among others but are **not compatible with ketones (e.g., acetone, MEK, ethyl acetate), low molecular acids, amines, highly polar chemicals etc**. They are **resistant to weathering and heat**, but for water samples the recommended max. temperature of operation is + 80°C.
- **The closures don't need to be disassembled for cleaning**, unless the specific SPME application results in carryover. The assembled Vac closure with O-rings and septum can be wiped or washed with e.g., ethanol, isopropanol, water, and water containing liquid detergent, and then oven-dried if necessary. **It is reminded that acetone can be used for washing the metallic body of the closures but not the O-rings.**
- The condition of the septum and O-rings is critical for preventing vacuum deterioration/loss. **The septum of the Vac closure can afford up to 100 penetrations, although this may vary.** The O-rings have a much longer lifetime, and their replacement is needed less often. Any unusual resistance found when fitting the closure to the vial may be a sign of deteriorated O-rings that should be replaced.

5. Troubleshooting

- **IMPORTANT NOTE:** When using an autosampler, **setting an appropriate vial insertion depth is critical**. A correct value for this parameter ensures full exposure of the SPME Fiber or Arrow extracting phase to the headspace. At short vial insertion depths, the SPME Fiber or Arrow phase can be in continual contact with the closure's septum during extraction. This will erode the extracting phase and render SPME Fiber or Arrow inefficient. **At an initial stage, the vial insertion depth must be determined and used thereafter.**
- The loss of vacuum cannot readily be detected during Vac-SPME sampling; however, once sampling is finished, and the pressure inside the sampler is equilibrated with atmospheric pressure, the vacuum relief sound is significantly lower when the vacuum is deteriorated. In some extreme cases when there is a complete loss of vacuum, there is no sound at all. In case of vacuum loss, the resulting Vac SPME signals become similar to those obtained with regular SPME.
- With Vac-SPME, high extraction efficiencies and very good sensitivities can be achieved at mild temperatures. This feature eliminates the need for heating the sample at elevated temperatures as seen with regular HS-SPME. The advantages of sampling at mild temperatures include preserving the volatile profile of samples and minimizing matrix effects for complex samples. SPME fibers are low-capacity sorbents, and at high sampling temperatures, water molecules can interfere with analyte uptake by the SPME fiber, especially when absorbent-type SPME fibers are used (PDMS coating). **When using this fiber, elevated temperatures should be avoided when sampling water or water-containing samples as they might reduce the performance of Vac-SPME Fiber methods. This effect is not recorded when using a high-capacity sorbent like SPME Arrow. If elevated sampling temperatures are necessary, we recommend switching to Vac-SPME Arrow.**

6. Use of Vac-SPME Fiber and Vac-SPME Arrow closures with Centri extraction & enrichment platforms

Use of Vac-SPME Fiber and Vac-SPME Arrow closures can be automated with Markes International's Centri platforms. Full instructions for the Centri platforms can be found in the user manual, QUI-1144.

6.1 Robot teaching

Vac-SPME closures have a narrower aperture than industry standard crimp- or screw-type caps, requiring **stringent robotic teaching** to ensure the extraction tool (headspace syringe, SPME Fiber or SPME Arrow) pierces the septum in the correct position when introduced to the vial. **If teaching is incorrect, the extraction tool may instead strike the metal area on the top of the cap, causing the tool to bend and become unusable**. This affects all modules in which extraction tools may be introduced to vials, *i.e.*, Centri agitator, PAL3 agitator, Heatex stirrer, tray plates and tray holders.

6.2 Centri agitator, PAL3 agitator and Heatex stirrer

When using Vac-SPME caps with Centri, it is strongly recommended that the teaching of the Centri agitator, PAL3 agitator and Heatex stirrer are checked with a vial capped with a Vac-SPME closure. If there is any doubt about teaching precision, these modules should be retaught with the Vac-SPME capped vial.

6.3 Tray holders with R60 sample racks

When sampling directly from R60 vial trays, it is strongly recommended that the teaching of the tray holder module(s) with a Vac-SPME capped vial be checked. If there is any doubt about teaching precision, these modules should be retaught in the usual way.

6.4 Tray plates / holders with VT15 sample racks

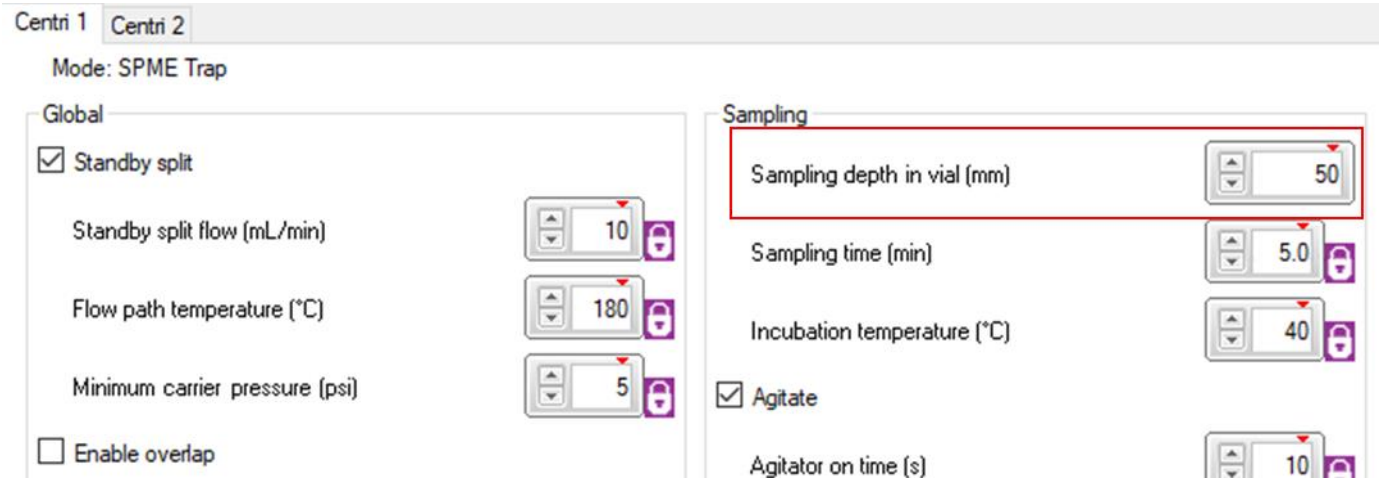
When sampling from VT15 sample racks, additional teaching steps are required before first use of Vac-SPME capped vials. From the virtual terminal home screen, select the tray plate / holder and then hold 'A' and 'B' together on the PC keyboard to access and select 'Extended User Level'. Select 'Slot 1' > 'Rack' > 'Options' > 'Teach PALmodule'. Complete the following four step process:

1. Place a vial with a Vac-SPME cap fitted into **position 1** (back left) of the VT15 sample rack situated in slot 1 (back slot) of the tray plate / holder. Move the mounted tool above and onto the vial so that the bottom of the tool is aligned centrally with the vial. Select 'Save'. You will then have the option to manually adjust the teaching position. Select 'Next' if no manual adjustment is required.
2. Place a vial with a Vac-SPME cap fitted into **position 11** (front left) of the VT15 sample rack in slot 1 (back slot) of the tray plate / holder. Position the mounted tool onto the vial so that the bottom of the tool is aligned centrally with the vial. Select 'Save'. You will then have the option to manually adjust the teaching position. Select 'Next' if no manual adjustment is required.
3. Place a vial with a Vac-SPME cap fitted into **position 15** (front right) of the VT15 sample rack in slot 1 (back slot) of the tray plate / holder. Position the mounted tool onto the vial so that the bottom of the tool is aligned centrally with the vial. Select 'Save'. You will then have the option to manually adjust the teaching position. Select 'Next' if no manual adjustment is required.
4. Select 'Ok'. The mounted tool will move back to the home position. Return to the tray plate / holder screen in the virtual terminal. Repeat the above steps for 'Slot 2' and 'Slot 3'.

6.5 Centri method setup

SPME Fiber and SPME Arrow Centri methods

The septa used with Vac-SPME caps are significantly thicker than conventional septa, hence SPME fiber and SPME Arrow tools must be inserted deeper into the vial such that when the needle is retracted, the entire length of sorptive phase is exposed correctly for extraction. When setting up a 'Direct SPME', 'Direct SPME Arrow', 'SPME Arrow Trap' or 'SPME Trap' method, the **'Sampling depth in vial (mm)'** parameter must be set to at least **50 mm**. **Failure to carry out this step will mean some of the sorptive phase remains in the septum during sampling and will potentially be stripped when retracted.** All other parameters can be selected as normal.



Headspace methods

When using Vac-SPME caps with 'Direct Headspace' or 'Headspace Trap' methods, the 'Sampling depth in vial' parameter should not be set below the default 25 mm.

More information

For technical support, please contact ExtraTech in the first instance. If they are unable to resolve your query, please contact Markes International's service department.

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