GETTING STARTED WITH HEADSPACE ANALYSIS

SYSTEM STARTUP ESSENTIALS

The CTC Combi Pal is a versatile system that can be used for many sample preparation and autosampling tasks. As such it is great for automated headspace analysis. In order to take full advantage of the system, there are a few points to consider before starting automated sample extraction and loading.

HEADSPACE GUIDE FOR Combi Pal

1. Headspace vials, septa and caps.

The single most important contributor to bad reproducibility for headspace GC results is leaky vials. The only recommended vials from the manufacturer are the screw-top vials from La-pha-Pack. The vials (packs of 100) can be purchased from AUTOSAMPLERGUYS, part number: 1809 1306 (10 mL) or 1809 1307 (20 mL) and caps (packs of 100) are available with either butyl rubber/PTFE septa (1803 1416), low background Viton septa (1803 1424) or ultraclean silicone/PTFE (1803 1414 and 1803 1309). As a result of the high sensitivity possible with large volume headspace injection, septum bleed in the sample vials during sampling may result in significant background during the run. Sometimes it is worth baking out all septa before use. This can be done in an incubator or in the GC oven. Use moderate temperature but allow several hours for the bake-out.

2. Needle penetration.

The headspace syringes have a side-hole needle. In some cases, the hole is up to 5 mm up from the very tip of the needle. Therefore it is important that the needle penetration depth in the vial is set well below the septa. For the Split/Splitless and the PTV[1] injector from Agilent we recommend a depth of 54mm. The Varian inlet requires an injection depth between 45 and 50 mm. The PTV and the Split/Splitless injector of Thermo Finnigan Trace GC require an injection depth between 45 and 50 mm. The Unis from JAS requires an injection depth between 35mm and 45mm.

3. Clogged needle.

A clogged needle would prevent sample to be picked up from the vial and results in a lack of response and/or poor reproducibility. Testing the syringe by hand if it is clogged is imperative.

[1] *Please note that the Agilent PTV is the same inlet as the Gerstel CIS4.*

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Feel if the needle side hole is smooth. If it feels rough or sharp use a fine grade sand paper or Emery board to grind any sharp edges. This will prevent coring of septa. Particles may have collected in the bottom of the needle tip. Wash out the syringe and needle and then blow it out with a dry gas.

4. Plunger speed, filling strokes, delays in sample vial.

Picking up sample from the headspace vial is an extraction process that is different from picking up liquid sample; it should be done rather slowly; the larger the sample the slower the plunger speed. A delay of 5-10 seconds should be following the extraction.

5. Temperatures and agitation.

Choose the lowest feasible temperatures; e.g. no need to go above 45 degrees for blood alcohol work. If you use the syringe at a higher temperature (80°C and above) be beware that the syringe might not seal any more at lower temperatures. The Teflon of the plunger will soften (deform) and the syringe should be marked for high temperature use only. During an application, keep the syringe temperature 5 degrees higher than incubation temperature. Make sure agitation does not create such high liquid turbulence that the septum gets wetted from inside the vial.

6. Salting out

For increased headspace concentration, the addition of a salt such as NaCl is recommended. Typically, 20 %w/v is sufficient. Salt helps to increase the partitioning of volatiles into the headspace of the vial.

7. Injection of headspace gas sample.

Adjust the injected sample volume to get sufficient response, but no peak tailing. Sharp chromatograms are in most cases a function of sample volume and injection speed; separation problems are in most cases a function of the GC oven temperature cycle and the carrier gas flow. For sharper peaks, increase the injection speed. For good chromatography it is essential to find a balance between volume and injection speed.

8. Choice of inlet liner.

This can have a significant effect on the quality of the chromatography. If injections will be made into a hot inlet, a straight liner is recommended. If injections will be made into a cool inlet while analyzing volatiles and semi volatiles, it is preferable to use an inlet liner packed with acid washed silanized glass wool or quartz wool, sometimes glass beads work better. For very low boiling compounds like vinyl chloride, chloromethane or one of the Freons, a Carbotrap packed liner is usually preferred.

9. Headspace Syringe handling.

The following headspace syringes are available: 1mL, 2.5 mL and 5mL syringe. Syringes are consumables and need to be replaced frequently. Often, plunger replacement might be more cost effective. When using a new syringe, make sure that the plunger moves easily up and down the syringe barrel. If it is very hard to do so, bake the syringe for 2 hours at 35 °C in the GC oven. **Do not exceed this temperature**, or the syringe will only seal at high temperatures.

10. Literature.

This Guide assumes familiarity with the fundamentals of headspace method development. Beginner users should refer to a fundamental text on headspace analysis such as *Static Headspace-Gas Chromatography Theory and Practice,* by Bruno Kolb and Leslie S. Ettre, Wiley-VCH (1997).

SETTING UP THE Combi Pal FOR HEADSPACE ANALYSIS

Note: Headspace needles can be damaged while mounting and dismounting the syringe holder adapter in the injection unit. We recommend mounting the holder without a syringe installed to practice aligning the locating pins in the injection unit.

To install the headspace syringe 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 plunger holder. All syringe holders and plunger holders have a color code.

Press the lower needle-guide upwards, so that it sits inside the upper needle-guide. Carefully install the headspace syringe holder by first inserting the needle through both upper and lower needle guides. After the needle passes smoothly through both needle guides, raise the headspace syringe holder until the lower locating pins snap into their corresponding holes in the injection unit. The top of the headspace syringe holder then will snap magnetically into the injection unit. Secure the headspace plunger with the red plunger holder. If syringe auto-detection is turned off, enter the syringe that is installed in the injection unit.

When preparing to run headspace methods with the Combi Pal, there are a few parameters that must be modified using the keypad. **Caution! Use care in teaching or checking positions of objects in the Combi Pal if the headspace syringe is mounted in the injection head. If incorrect coordinates are set, and the injection unit drives the needle downward into an object, the needle can be irreparably damaged.** If many object positions must be taught (as when setting up a new system) it is safest to remove the headspace syringe holder first.

The Combi Pal is preset with default parameters for injector penetration (40mm) and vial penetration (22mm). The injector penetration depth has been optimized for use with liquid or

SPME injection for most inlets, and should be reset through the "Utilities" menu on the keypad. Go to Utilities/Injectors/Injector Penetration and set needle penetration to 25mm. The vial penetration default is appropriate for headspace analysis depending on choice of vial size (10ml or 20ml) and sample volume.

The Combi Pal can be set with default temperatures for the syringe and agitator that keep these objects warm between samples and minimize heating time. These default settings are found in the Utilities menu on the keypad. Always set a default temperature lower than the lowest temperature to be used in a series of runs, since cool down is very slow on these objects.

TYPICAL HEADSPACE METHOD PARAMETERS

It is impossible to provide general recommendations for headspace conditions appropriate for all analyses. The following recommendations and comments have been developed based on analysis of a range of compounds seen in the headspace at 60°C above a variety of sample types.

Agitator Temperature - Set to temperature appropriate for sample

Syringe Temperature - Set 1-5°C hotter than agitator to prevent condensation

Syringe Fill Speed - Typically 50-200 µl/s

Syringe Fill Strokes - Typically 1-2 strokes (only an option while using Cycle Composer software)

Pullup Delay - Allows pressure in syringe and sample vial to equalize, typically 2-5 seconds

Injection Speed - For cool inlets, slow injection speeds e.g. 20-100 µl/sec usually better; for hot inlets try 300-800 µl/s.

Pre Inj Delay - Typically short e.g. 200 ms

Post Inj Delay - Typically short 200-500ms

Syringe Flush Time - Depends on sample concentration; typically 2-4 minutes, pressure at 1 bar

GC Runtime - also known as cycle time from ready to ready
GC oven program + oven cool down cycle + GC equilibrium time

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