Appendix VII: Experimental manual and troubleshooting guide

See Appendix VIII for some pictures of the process.

Pyrolysis-GC/MS procedure for paint samples
(developed for samples of art and archaeology)

Marie-Louise Tambo Magni, NTNU, 30.09.2019

A. Preparing and closing the system

B. Procedure when running samples

C. Extensive cleaning

D. Troubleshooting: Sample handling, sample runs and cleaning procedures

Final procedure for Pyrolysis-GC/MS analysis

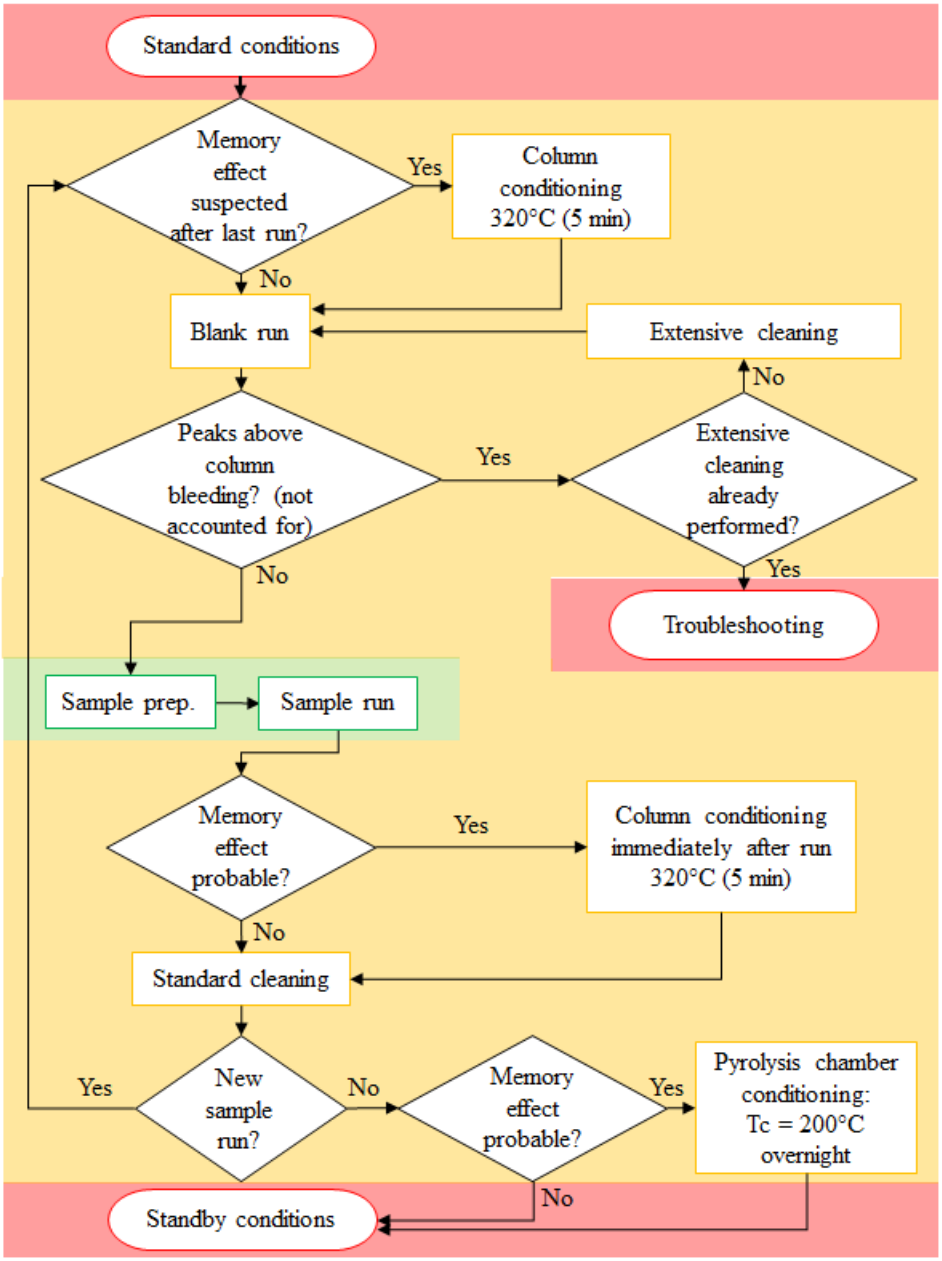

Figure: Final procedure for pyrolysis-GC/MS analysis. Flow chart of the setup for pyrolysis-GC/MS procedures in the last batches (Batch 5 and 6). Circles (○) indicates starting and ending points, diamonds (◊) indicates decisions and squares indicate actions (□). Red indicates steps where the experiment is started or put on halt, yellow indicates steps performed before and after the actual analysis of the sample aiming to improve the reproducibility and green indicates actions related to actual analysis of a sample.

A. Preparing and closing the system

1) Preparation of system (when starting up the system for the day)
    a. Set injector/inlet, transfer line/auxiliary line, He-flow and T_c to standard conditions.

2) System standby (when ending analyses for the day)
    a. Set injector/inlet, transfer/auxiliary line, He-flow and T_c to standby conditions. T_c can be set to 200 for conditioning overnight if persistent memory effect/contaminations are suspected/detected

B. Procedure when running samples

1) Run blank (empty filament)

Note: Ensuring a clean system can be time consuming. The first blank of the day is expected to contain non-acceptable chromatographic peaks (high intensities and high bleeding may occur). For the first runs after a period of inactivity several blanks can be needed.

Preparation before blank run

a. If not done after preceding sample run, or if first run after inactivity:
    i. Flame clean filament
    ii. Flame clean glass cell. Let it cool on ceramics for a couple of minutes (while e.g. cleaning filament further or setting up next run). System only enclosed while glass cell is inserted; insert glass cell when ready.
b. Before first blank of the day, if contaminations/memory effect suspected:
    i. Column conditioning 320 °C (5 min)

Blank run:

c. Add "file name", "path" and "inst. meth." in sequence setup
d. Menu -> Actions -> Run This Sample
e. Reassure the right sample setup is to be run (check Row#)
f. Press "no" to the pop-up (or yes. It doesn't really matter when not running sequences)
g. Wait for both Trace GC Ultra and ITQ 1100 to say "Ready for Run"
h. Press 
i. Wait for "Waiting for contact closure" on both Trace GC Ultra and ITQ 1100
j. Manually press "Start" on GC and "Start Py" in ISO setup in Pyrola 2000 simultaneously

2) Sample run

Preparation

a. Preparation in Xcalibur: Add “file name”, “path” and “inst. meth.” in sequence setup
b. Turn He-flow **S/SL<-Py**
c. Replace filament with dummie-probe (Beware! The glass cell can follow when taking out the filament. Use tweezer to carefully put it back in).
d. Flame clean filament
e. Add sample to the cavity in the middle of the filament
f. Add derivatisation agent or water if needed (be aware to finish step g-j during desired reaction time)
g. Insert filament in pyrolyser
h. Turn He-flow **S/SL->Py**
i. Ensure that pressure is stable and perform leak check when unsure of system enclosure
j. Run sample. *Same procedure as running blanks (1d-j):*

3) Cleaning procedures (after sample run)

a. Turn He-flow **S/SL<-Py**
b. Take glass cell and filament out and replace filament with dummie (Beware! The glass cell can follow when taking out the filament. Use tweezer to have control over it)
c. Flame clean glass cell. Let it cool on ceramics for a couple of minutes (while e.g. cleaning filament further or setting up next run). System only enclosed while glass cell is inserted; insert glass cell when ready.
d. Flame clean filament
e. If persistent stains are present, or if risk of memory effect is high: Clean filament and/or glass cell further with water and acetone with cotton swaps.
f. Insert glass cell and filament in pyrolyser
g. Turn He-flow **S/SL->Py (**Helium flow should go through the pyrolysis unit, S/SL->Py, when not in use)

C. Extensive cleaning

Memory effects, when intensities of chromatographic peak >10^7 cps in sample run:

a) Column conditioning (325°C for 5 min) directly after sample run.
b) Condition pyrolysis chamber at 200°C overnight.
c) Column conditioning (325°C for 5 min) before first blank of next day of analysis.

Contaminants (unknown peaks, not obviously coupled to previous runs)

Quarts cell, filament, filament holder, helium conduit and septa examined.

a) A new round of flame cleaning of quarts cell and filament.
b) Wet cleaning (water and acetone) of quarts cell, filament, filament holder, helium conduit (and septa, if visual stains present. Should be avoided elsewise, since septa can absorb acetone creating other interfering peaks in the chromatogram).
c) Careful mechanical cleaning of filament holder when stains persisted both flame and wet cleaning. Persistent inorganic (white) stains on filament was ignored.
d) New short round of flame cleaning of quarts cell and filament to remove possible remains of water or acetone.

D. Troubleshooting: Sample handling, sample runs and cleaning procedures

To ensure reproducibility, system must be calibrated when new parts (glass cell, filament, septa, column etc.) are replaced or readjusted, and when R_0 or TTP differs significantly from last calibration.

Note:. For problems related to the equipment in general (Pyrola 2000, Pyrol AB or GC Trace Ultra ITQ1100, Thermo Scientific), troubleshoot according to their corresponding manuals.

The explanations and solutions are only suggestions and based on samples of cultural heritage (organic samples: egg, glue, oil, wax, fatty acids etc.)

Sample handling

Problem	**(Suggested) explanation and solution**
Sample blows off before pyrolysis (is found inside the glass cell)	a) Disperse in suitable solvent (water?)/use solvent as "glue" b) Lightly create a (deeper) cavity in filament (recalibrate if necessary) c) Lightly reposition filament or filament holder (recalibrate if necessary) for sample and helium flow crossing each other at another angle. d) Squeeze sample as flat as possible to increase contact surface with filament. e) Consider using a capillary tube sample handling option instead.
Strong memory effects running reference samples	a) Extensive cleaning (worst case: clean MS) b) Dilute next time to avoid these problems!
Low intensity of peaks	a) Add more sample b) Evaluate whether sample blows off before pyrolysis c) Raise t_2 to e.g. 5 or 10 sec (more time thermal degradation)

Sample run/analysis

Problem	**(Suggested) explanation and solution**
ITQ1100 or Trace Ultra is not running when pressing start or not getting ready	a) Forgot pressing “play” -> forgot waiting for “waiting for contact closure” b) Tune opened c) “start when ready”/change settings for instrument start.
58 m/z “interference”	TMAH fragment overall present
My sample run looks blank	Sample probably not present on filament during pyrolysis (or has vaporated before insertion in pyrochamber/very volatile?): a) Sample has blown off in chamber or before insertion (see sample handling) b) If liquid added to solid sample with needle/tools have been in contact with sample: Sample can have followed the tool leaving only the liquid on the filament.

Cleaning procedures and miscellaneous

Problem	(Suggested) explanation and solution
Blank non-acceptable (contaminations, memory effect or systematic)	First blank of the day a) Run new or b) Consider conditioning possibly affected parts, then run 1-2 blanks c) Consider conditioning before first blank if the problem is repeating. Ghost peaks (System related) a) Have septa recently been replaced? Changing septa usually cause a spike in ghost peaks, as some of the ghost peaks originates from septa/softeners System overload/memory effect a) Extensive cleaning procedures: wash filament, filament holder and glass cell thoroughly, long conditioning of pyrolysis chamber, condition of column. b) If persistent: wash septa in pyrolysis chamber or change septa in pyrolysis chamber if it seems to have absorbed the component causing the memory effect (this will give rise to strong ghost peaks from the septa/septum bleeding, which can be ignored if eluting at a time not interfering with the samples).
Weird shape of TTP	a) "wave": Filament/filamentholder/skrews can be loose or bent in off direction, tighten/bend (carefully!) (recalibrate if R0 or TTP is off) b) "Oscillating"/zigzag: Optic cable might be a bit loose (BEWARE: metal is same temperature as pyrochamber!) c) Calibrating at 600: calibrate for a temperature a bit higher (620?), TD is difficult at 600.

R0 close to 100	Filament is probably used up, TTP probably difficult to reproduce over time: change filament.
Septa in chamber is stuck/difficult to take out during replacemen	Use special tool from Pyrola (see drawing in box).