WINEPOLL PROJECT.

2nd MANAGEMENT MEETING.
Ljubljana, Slovenia 26th February 2013.

DESCRIPTION OF WORK PROGRESS: WORK PACKAGE 1.

DETECTION OF MUSTY TAINT AGENTS IN WINE AND WINERIES.

TASKS IN WP 1

- **Task 1.1 DETERMINATION OF SAMPLING METHODS**

  Combination of samplers – sorbents.

  a) Passive samplers. Radiello™ Cartridge Adsorbents for sampling Phenolic Compounds (thermal desorption), matrix SS net (100 mesh, 4.8 mm diam.), Tenax-TA.

  Radiello™ Cartridge Adsorbents for sampling Anaesthetic Gases/Vapors, matrix SS net with mix of mol sieve and activated charcoal (30-50 mesh).

  Radiello™ Cartridge Adsorbents for sampling BTEX and VOCs (thermal desorption), matrix SS net (3 x 8 µm, 4.8 mm diam.), Carbograph,

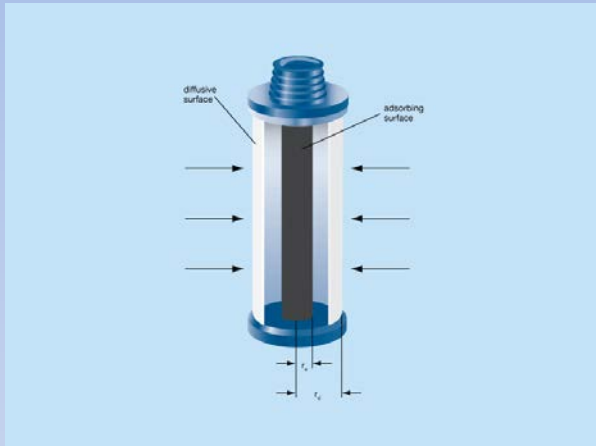
  b) Active samplers. Zambelli EGO Plus TT active calibrated sampler. Air flow 25 – 65000 ml/min.

  Sorbent Materials

  Carbopack B+X, Carboxen 1000.
  Carbopack C+B, Carbosieve SIIITM.
  Tenax GRTM.
  Carbopack B+X.
  Tenax TATM.

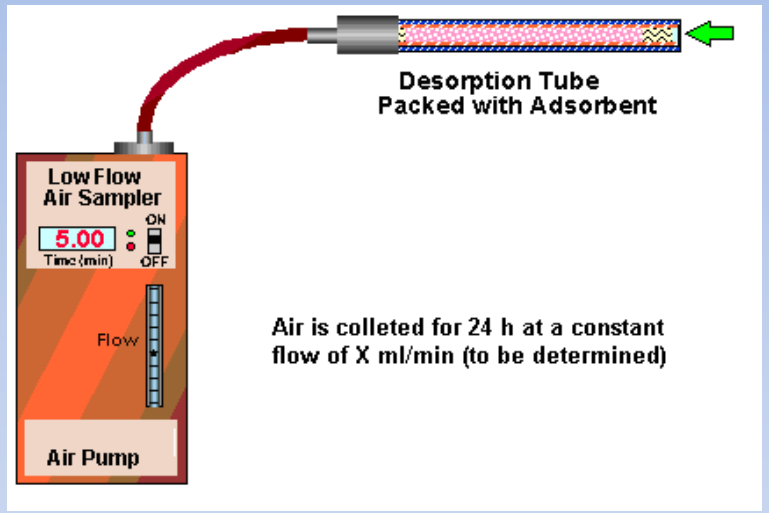
  .

What is a passive sampler?



- Static
- No need to generate air flow.
- Low cost.
- Long term air monitoring (> 24 h)
- Air monitoring in remote locations (no electricity).
- Low detection limits.
- Multiple samplers can be located at the same time and place with ease.
- Need of of calibration for specific uses.

What is an active sampler?



- Combination of air pump with controlled flow and sorbent material.
- No need for calibration of adsorbent.
- Fast sampling method.
- Multiple samples in a short period of time.

How do we measure the amount of absorbed compounds?

- Stage 1. Thermal desorption:

Use of heat in non-oxidant atmosphere (up to 350 ºC) to desorb the physically bound compounds in adsorbent material.

» Optimization of:

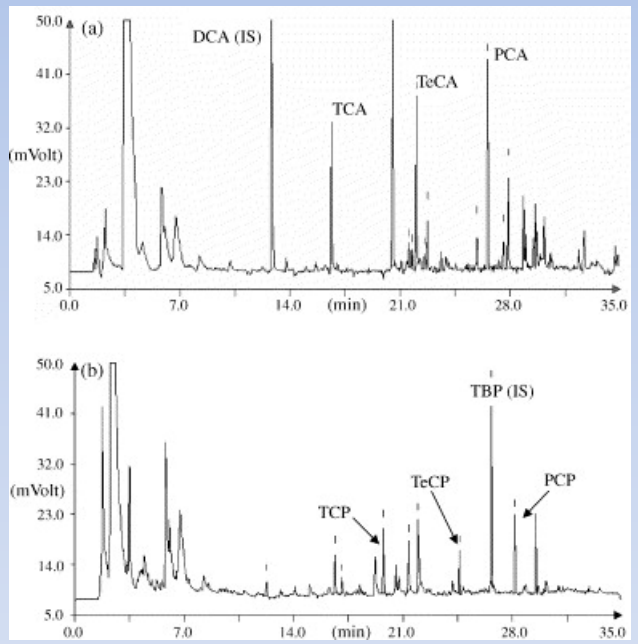
- Cryofocusing temp.
- Desorption gas flow.
- Desorption temperature.
- Temperature ramp.
- Transfer temperature.
- Desorption mode.

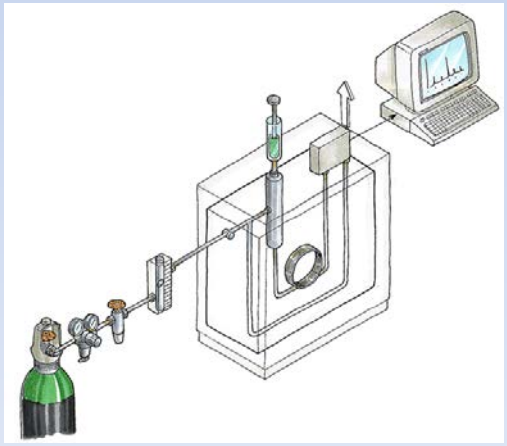
- Stage 2. Chromatographic Separation.

Chromatography

All chromatographic techniques flow the mixture, that is to be separated, through a material that **retains some components more than others**.

This causes different components to flow through the material at different speeds, so they separate.





Stage 2. Chromatographic Separation (cont.)

Optimization of:

Mobile phase. High purity He at different flow rates.

Stationary phase. Different capillar columns.

- non-polar column 5% phenyl.
- middle polar column 35% phenyl. Most adequate.

Solvent use. Mostly non polar solvents: cyclohexano, n-hexane,etc

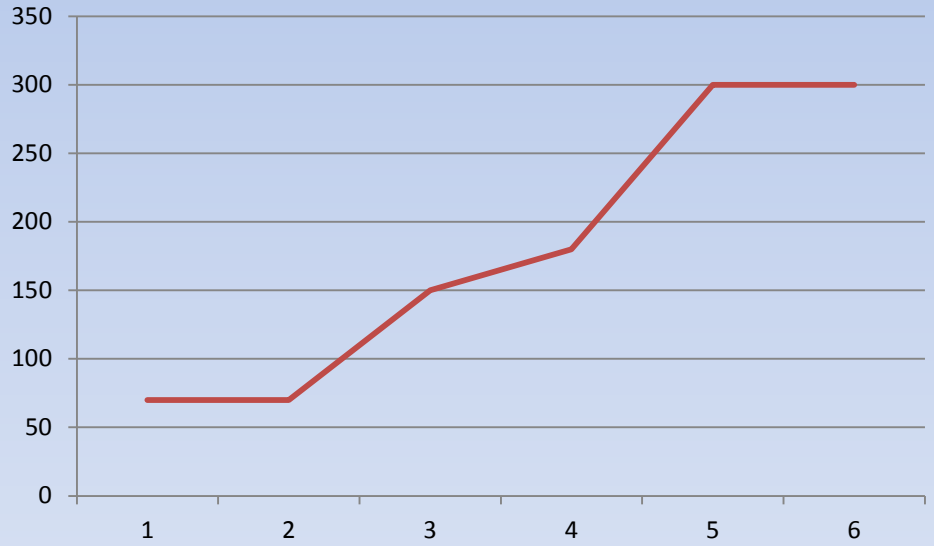
Injection mode. Splitless.

Injection volumen. 0.5 to 1.0 microliters.

- Stage 2. Chromatographic Separation (cont.)

Temperature ramp:

Oven temperature was 70ºC (held for 2 min).
70 to 150ºC at 25ºC min^{-1}
150 to 180º C at 3ºC min^{-1} to 180ºC,
180º C to 300º C at 25ºC
300ºC for 5 min.

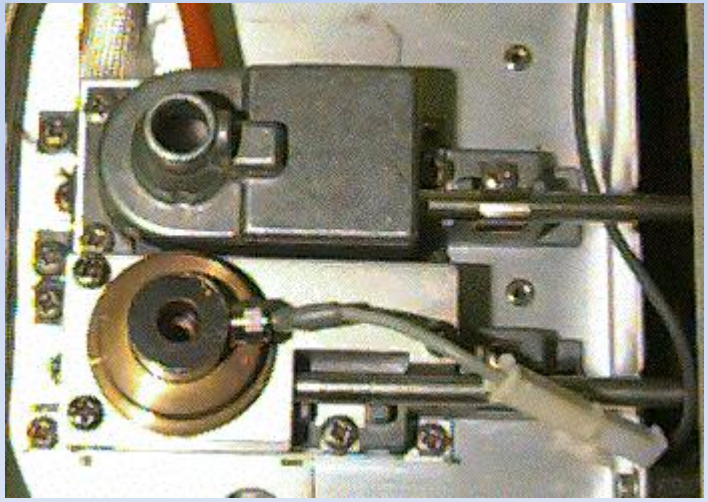


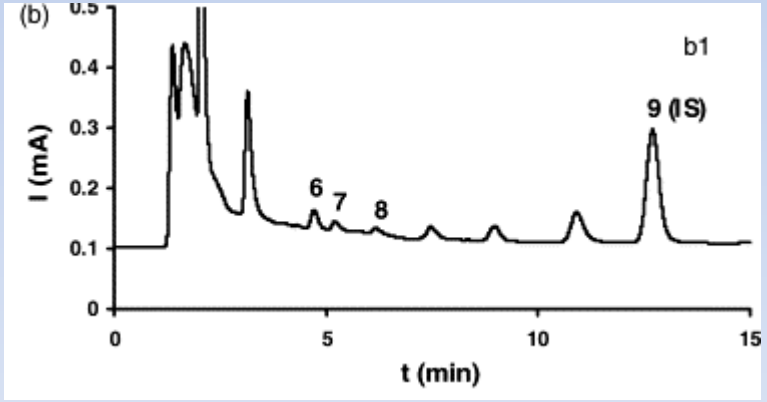
The inlet and detector temperature was 250 and 320 ºC, respectively.

- Stage 3. Compound detection.

- a) Electron Capture Detector.

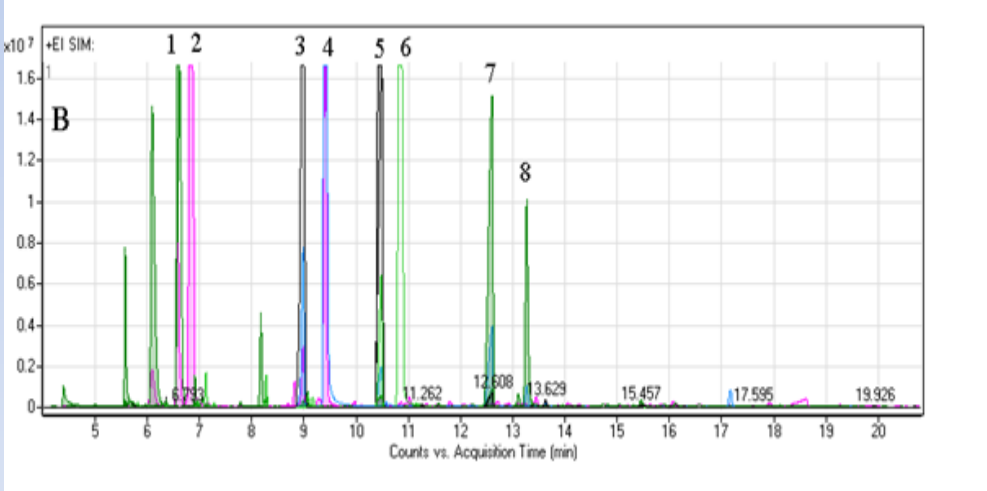
  - Selective to halogenated compounds (Cl, Br).
  - Confirmation needed.
  - Detector temperatura was set to 320ºC. Make up gas 40 ml/min

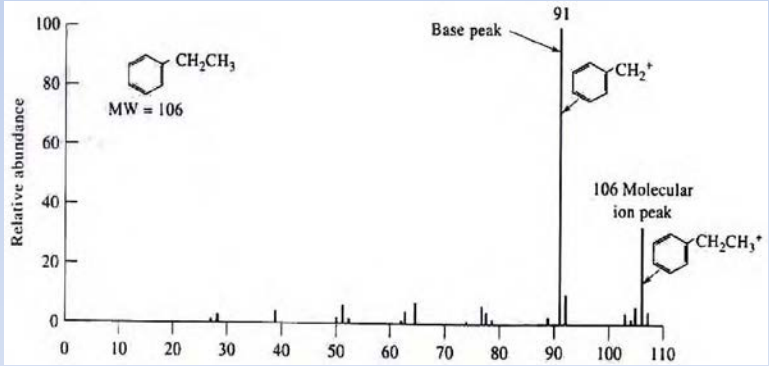




b) Mass spectrometer Detector.

- Molecular fragmentation. Measurement of m/z peaks and peaks intensity.
- Selection of molecular peaks.
- Selection qualifier ions.
- Optimization of collission energy.

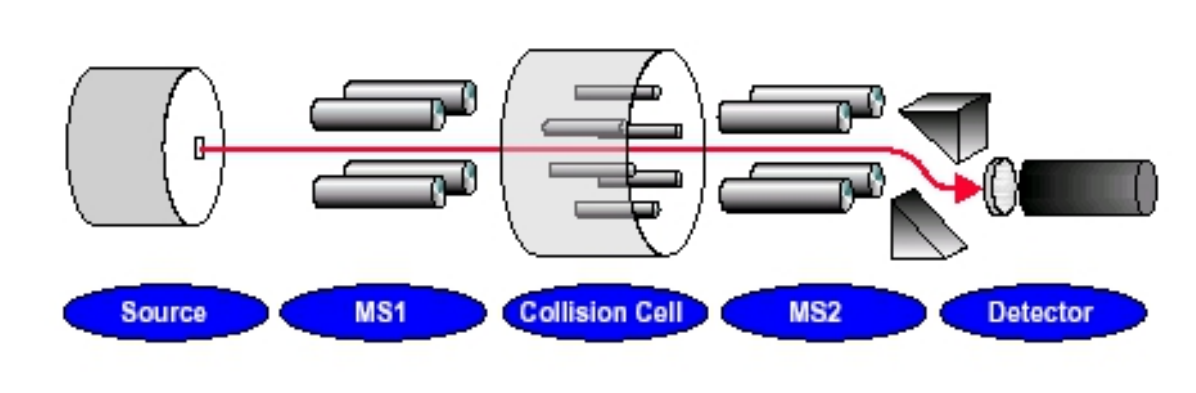




- Triple Quadrupole Mass Spectrometer Detector.

Molecular fragmentation and product ion fragmentation.

Stages:

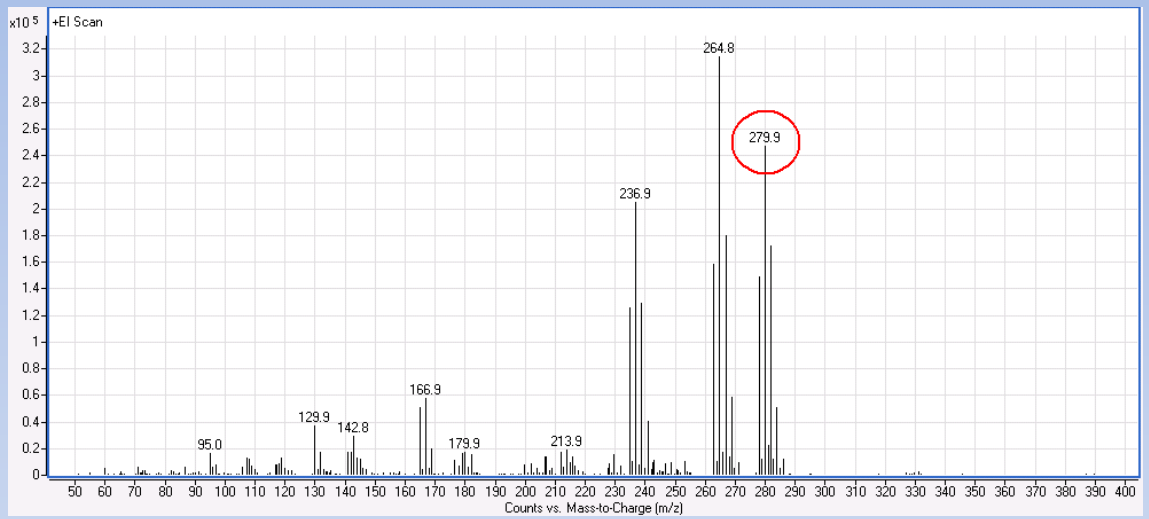


1) Analysis of the substances in Full Scan mode in order to obtain the MS1 spectrum and to select the precursor ion.

2) Analysis of the substances in product ion mode at different collision energies (CE) in order to obtain the MS2 spectrum and to select the product ions. At least two products ions must be selected.

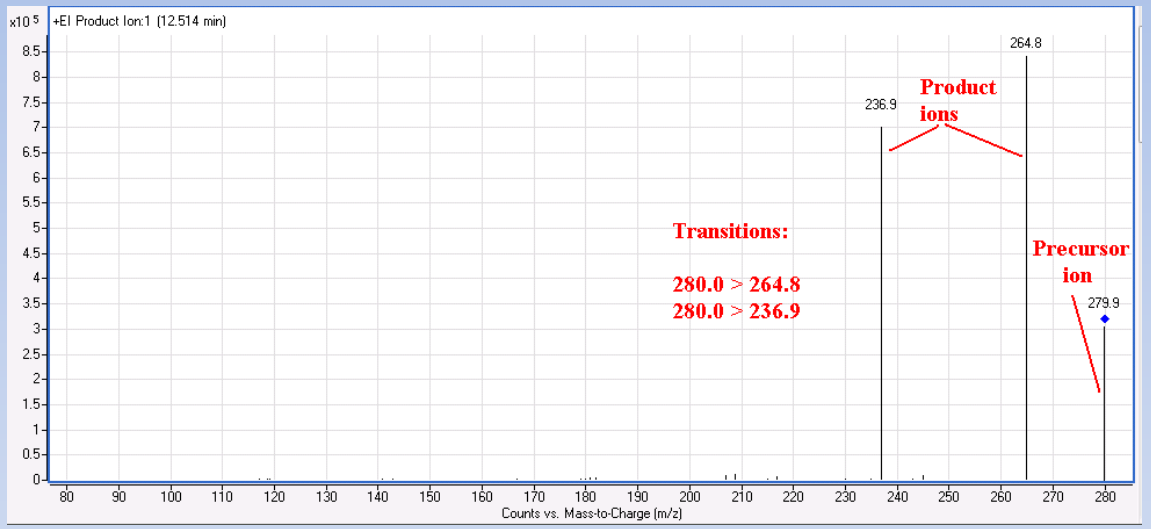
3) Analysis of the substances in SRM mode in all range of CE in order to optimize it and obtain the maximum ion intensity.

- Analysis of the substances in Full Scan mode in order to obtain the
- MS1 spectrum and to select the precursor ion.



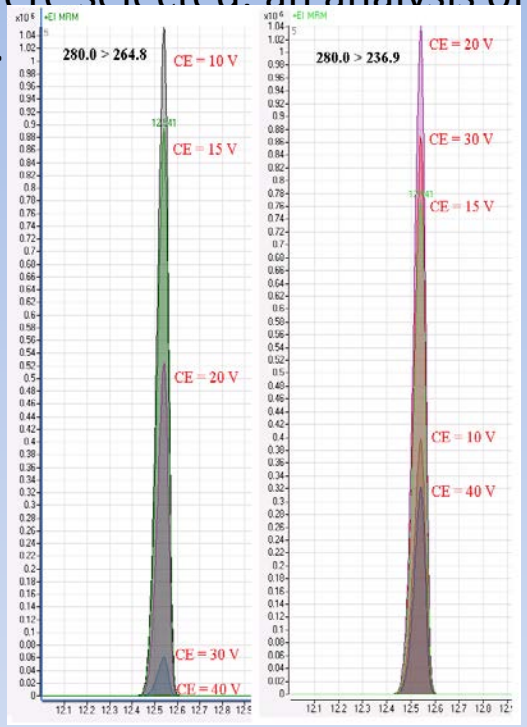
- >90% correlation with the reference spectrum. Ion 279.9 was selected because it has a good intensity and the highest *m/z* relationship. The selected *m/z* corresponds to the molecular ion. Good response and highest selectivity will be obtained.

- Ion 279.9 was broken in the collision cell and the resulting fragments were analyzed in product ion mode in order to obtain the MS2 spectrum and to select the product ions.



- Precursor ion 279.0 gave only two fragments, 264.8 and 236.9. They were selected as product ions and their optimal collision energy to obtain the maximum response was optimized.

- Once the products ions were selected, an analysis of the substances in SRM mode at different CE was made.



- The CE for which maximum response was obtained was selected for each transition. Finally, 10 V and 20 V were selected for transitions 280.0 > 264.8 and 280.0 > 236.9, respectively. Transition 280.0 > 264.8 was selected as primary because it has highest *m/z* relationship and has more specificity.

Table 2 summarizes the final working conditions for the triple quadrupole mass spectrometer

	Retention time (min)	Precursor (Da)	Product 1 (Da)	CE (V)	Product 2 (Da)	CE (V)	Product 3 (Da)	CE (V)
2,4,6-Trichloroanisole-d5	6,57	**215**	197	10	169	20		
2,4,6-Trichloroanisole	6,59	**210**	194,9	10	166,9	20		
2,4,6-Trichlorophenol	6,84	**196**	131,9	15	160	10	96,9	20
2,4,6-Tribromoanisole	9	**246**	230,9	10	202,9	20		
2,4,6-Tribromophenol	9,44	**232**	167,9	15	195,9	10	132,9	30
2,3,4,6-Tetrachloroanisole	10,45	**346**	330,8	10	302,7	30		
2,3,4,6-Tetrachlorophenol	10,87	**330**	249,8	15	221,9	20	247,9	15
Pentachloroanisole	12,63	**280**	264,8	10	236,9	20		
Pentachlorophenol	13,3	**266**	166,8	20	201,8	10	229,8	10

How do we measure the amount total concentration with active sampling?

- Calculation of total sampled volumen.

  Ej: 50 ml/min x 60 min/h x 24 h = 72 m3

- Calculation of total amount in sorbent materials.

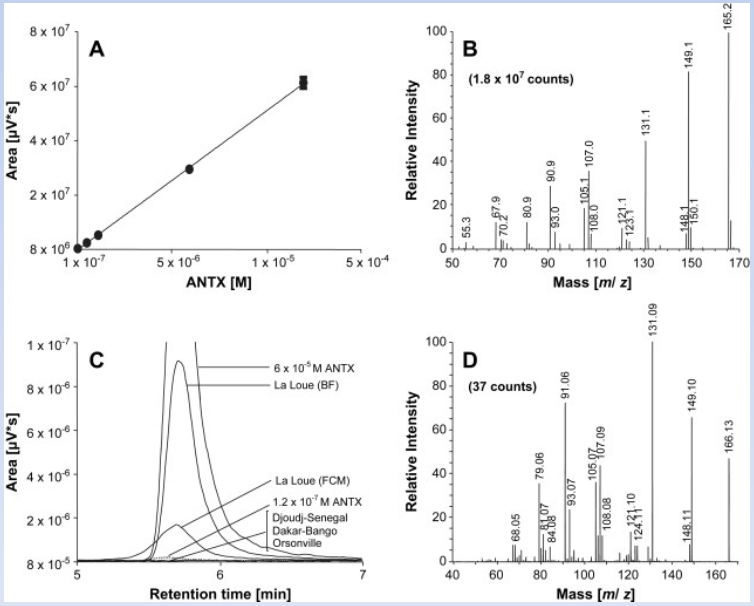
  From calibration curves of Gas Chromatography method.

- Calculation of recovery rates for sorbent materials.

  From spikes of known concentrations to sorbent materials.

Example:

- Volumen sampled in 24 hours: 72 m^3
- Recovery rate of 90 % for TCA in spiked Tenax tubes samples after thermal desorption when compared to pure solutions.
- 0.3 nanograms per Tenax Tube obtained from chromatographic method.



- Final concentration:

$(0.3/0.9)/72\ m^3 = 0.004\ nanog/m^3$

Odor threshold in air: 160 to 21000 ng/m^3.

Odor threshold in wine: 5 to 10 nanog/l.

How do we measure total concentration in passive sampling?

- Since no measured air is pumped through, there is a need to obtain a *mass transfer coefficient* Q for each type of sampler. This process is called passive sampler calibration.

- This mass transfer coefficient is compound and sampler dependant. Different coefficients need to be obtained for different types of passive samplers and different compounds.

- Once Q is determined for each analyte and passive sampler, the final concentration in the measure air can be determined as:

$$CA = M/(Q \cdot \Delta T)$$

Calibration of passive samplers

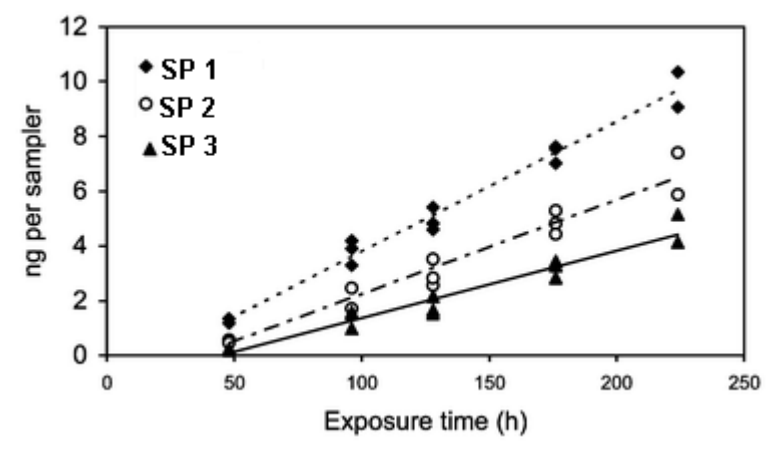
- At the same time the active sampling is carried out ,7 passive samplers of the 3 different sorbent materials (total of 21 samplers) will be deployed.

- At different times the passive samplers for the test chamber will be removed to obtain different adsorbed masses at different times. The period for this comparison will be 2, 5, 7, 10 and 15 days. At days 2 and 5, two samplers will be used in order to minimize errors derived from low mass detection.

- Passive sampler sorbents will be analyzed by TD-GC-MS/MS. The mass of HPs and HAs, in micrograms, in each sampler will be determined.

Calibration of passive samplers

- By plotting detected mass vs time of sampling we will obtain the Q values for each of the solvents.



- $M/CA = Q \cdot \Delta t$
- CA = Concentration in air. Fixed concentration with the use of sprayer. Determined with the use of active sampler.
- M = amount measured on each passive sampler with the use GC MS/MS method.
- Δt = Exposure time for passive sampler.

Calibration results

- This will yield the following results:

Radiello™ Cartridge type 1

	Qvalue
2,4,6-Tricloroanisol,	
2,4,6-Triclorofenol	
2,4,6-Tribromoanisol	
2,4,6-Tribromofenol	
2,3,4,6-Tetracloroanisol	
2,3,4,6-Tetraclorofenol	
Pentacloroanisol	
Pentaclorofenol	

Radiello™ Cartridge type 2

	Qvalue
2,4,6-Tricloroanisol,	
2,4,6-Triclorofenol	
2,4,6-Tribromoanisol	
2,4,6-Tribromofenol	
2,3,4,6-Tetracloroanisol	
2,3,4,6-Tetraclorofenol	
Pentacloroanisol	
Pentaclorofenol	

Continuing experiments

- Measurement method already set.
- Start of measurements of absorbed amount of TCA and TCP in passive samplers at different times under reproducible conditions with a fixed background concentration:
- Use of climatic chamber.
- Use of spiked humidifier to generate fixed air concentration.
- Use of active sampler to measure fixed air concentration.
- Selection of most active sorbent.
- Selection of most suitable combination sampler-sorbent.
- Expected results: 31 March 2013.