Green Sample Preparation Solutions based on Miniaturization and Automation.

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RIC technologies

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RIC technologies

21st Century Sample Preparation

- Keywords:
  - Automation
  - Miniaturization
  - Solventless (“green”)
  - High productivity
- Instrumentation: orders of magnitude increase in sensitivity using state-of-the-art MS (MS/MS, TOF...)

Sample preparation … automation

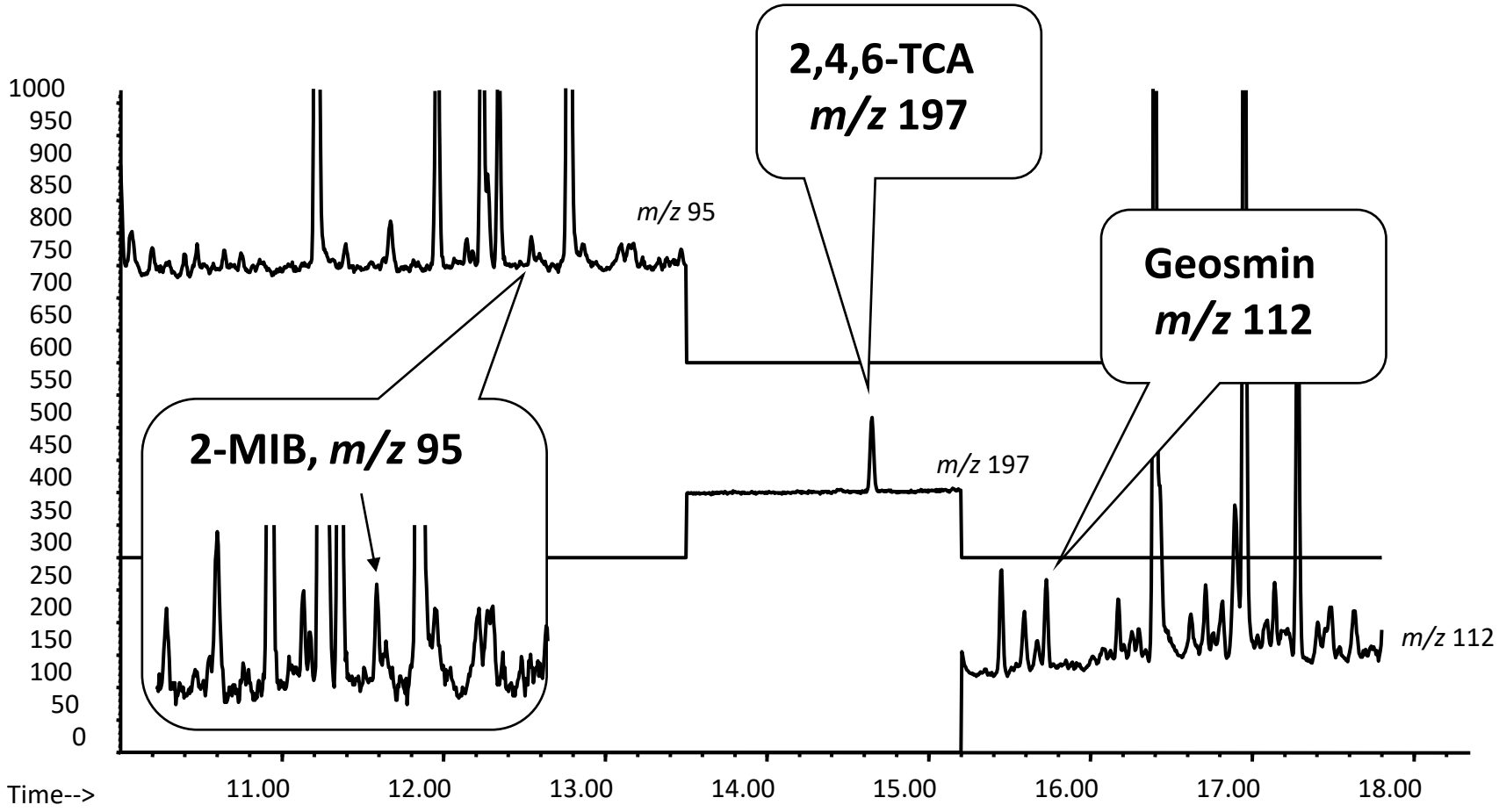
- Lecture: Two decades of Stir Bar **Sorptive Extraction**: a retrospective and future outlook.

1990: SPME (Pawliszyn)

1999: SBSE (Twister™, RIC – Gerstel)

More recently:
– SPME Arrow (CTC, Restek)
– HiSorb (Markes/SepSolve)

SBSE earliest application: musty odour in water



6 pg/60 mL = 100 ppq

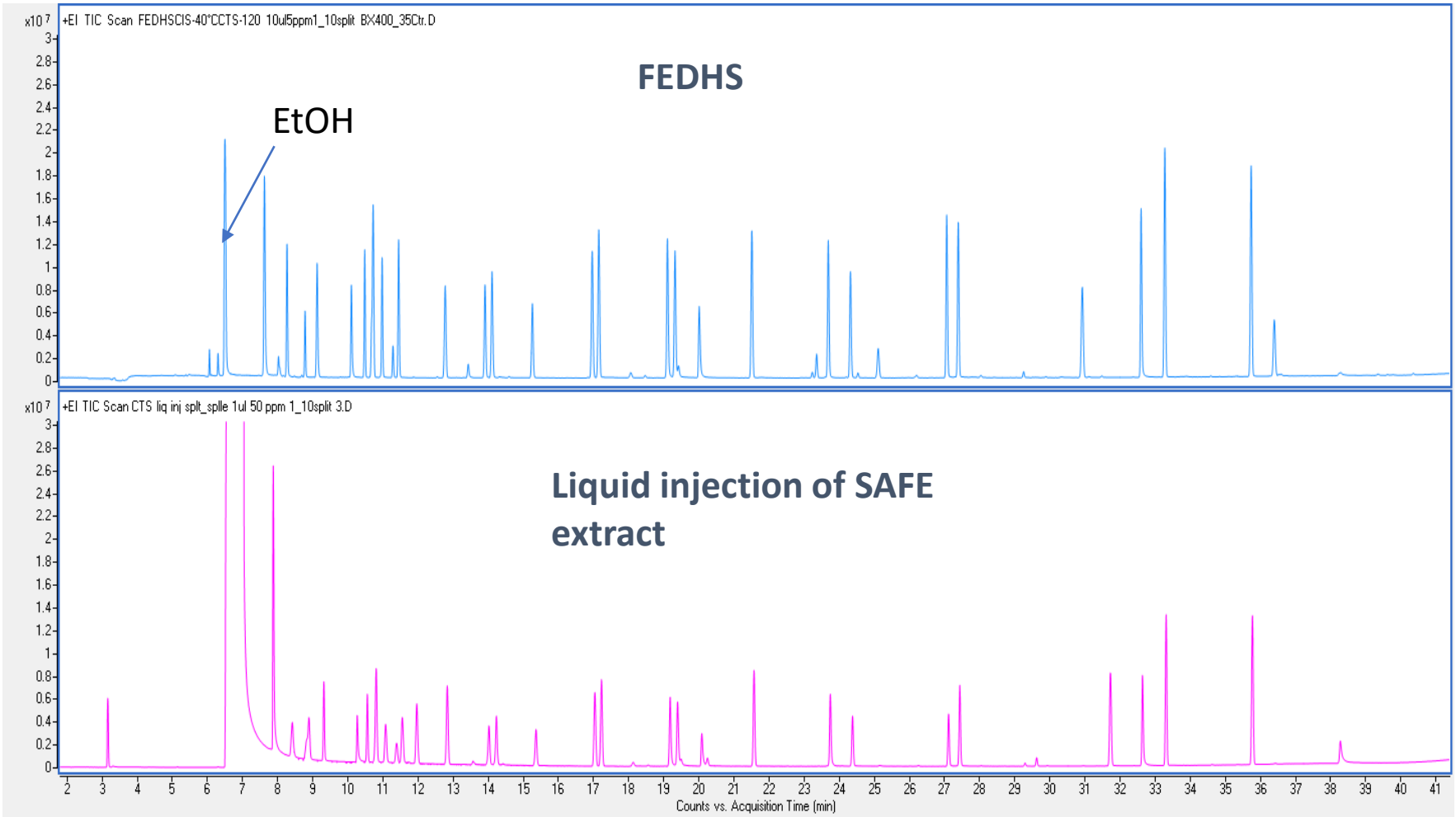
Aroma extraction: SAFE → DHS?

- SAFE: solvent assisted flavor extraction
  - Sample or solvent extract dropped in flask under vacuum
  - Vacuum destillation
  - Cold trapping
- No discrimination
  - Very volatile versus less volatile
  - Polar versus apolar
- Compared with steam destillation, SHS, SPME...DHS → FEDHS

Picture: https://research.reading.ac.uk/flavourcentre/flavour-analysis/flavour-extraction-techniques/

Engel, W., Bahr, W., & Schieberle, P. (1999). Solvent assisted flavour evaporation – a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. European Food Research and Technology, 209, 237–241.

Aroma extraction: SAFE → FEDHS



The 1999 Dioxin (PCB) crisis in Belgium

Ultrasonic Extraction

Matrix Solid Phase Dispersion

CGC - µECD

CGC - MS

To 5 mL extract 1 g SiO_2 containing 44% H_2SO_4 is added to remove the lipidic fraction

Method was developed … and published (in a book ☹)

- 50 samples per day/instrument
- Validation & accreditation
- > 5,000 samples analyzed at RIC

QuEChERS avant la lettre

The 1999 Dioxin (PCB) crisis in Belgium

21st Century Sample Preparation: challenges

- Very slow adaptation in industry and routine labs due to…
  - Complexity
  - Conservatism
  - Costs
  - Compromised results
- How to tackle?
  - 5 recommendations

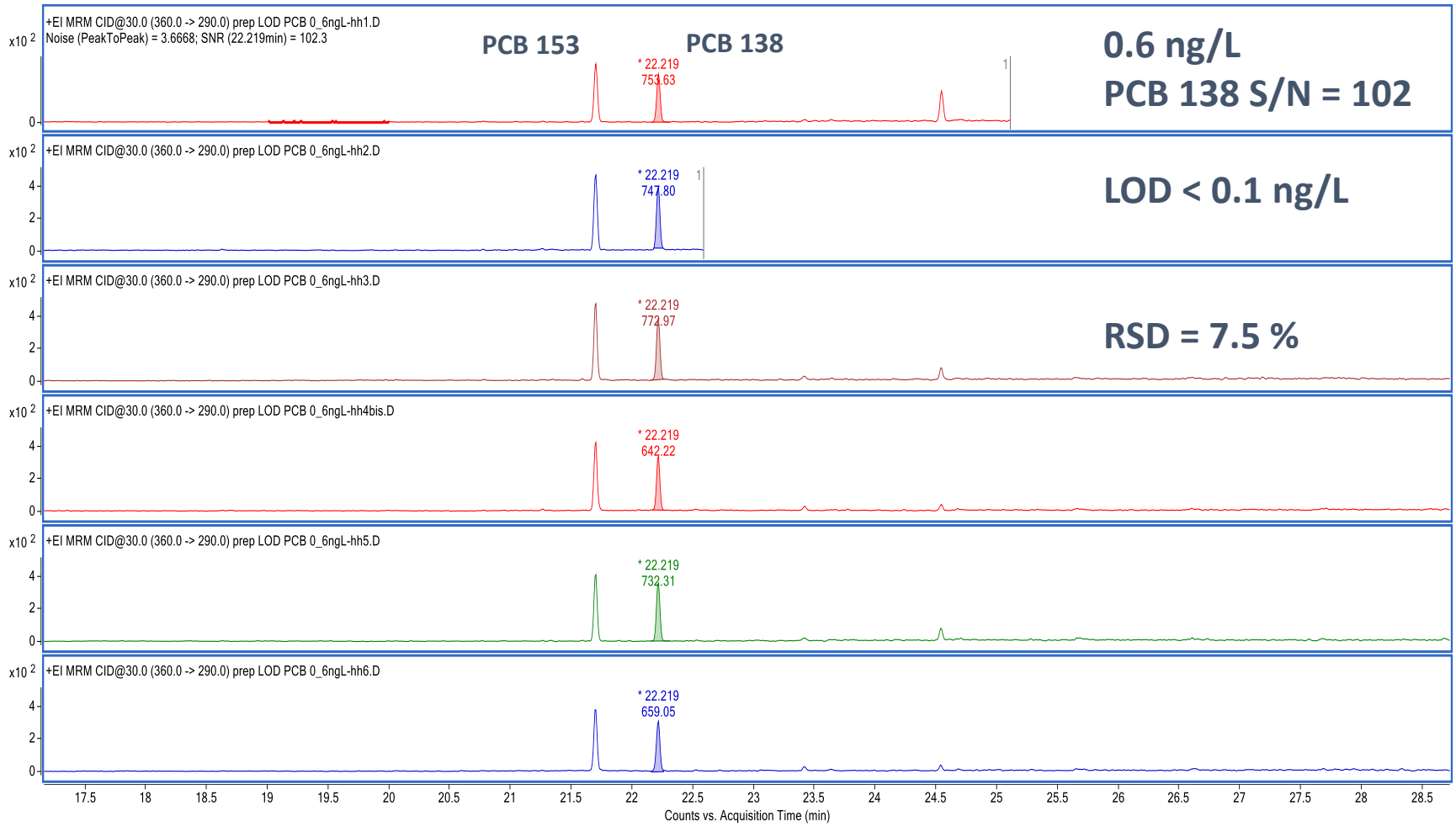
Importance of correct sampling and sub-sampling

POPs in surface water using automated µLLE - followed by LVI-PTV - GC-MS/MS

Reference method (ISO 28540)	Automated
1 L water + IS **Target LOD: 1 ng/L**	15 mL + IS **Target LOD: 1 ng/L = 15 pg/15 mL**
LLE with 25 mL hexane **100% recovery: 1 ng/25 mL**	µLLE with 3 mL hexane **100% recovery: 15 pg/3 mL**
Recover organic phase	Centrifuge (?)
Drying on Na_2SO_4	Transfer 1 mL to 2 mL vial with Na_2SO_4 **100% recovery: 5 pg/1 mL**
Concentration to 1 mL **Final concentration: 1 ng/mL = pg/µL**	Vortex & centrifuge (?) **Final concentration: 5 pg/mL = fg/µL**
GC-MS analysis 1 µL injection **Amount on-column: 1 pg**	GC-MS/MS analysis 40 µL injection (LVI-PTV) **Amount on-column: 0.2 pg**

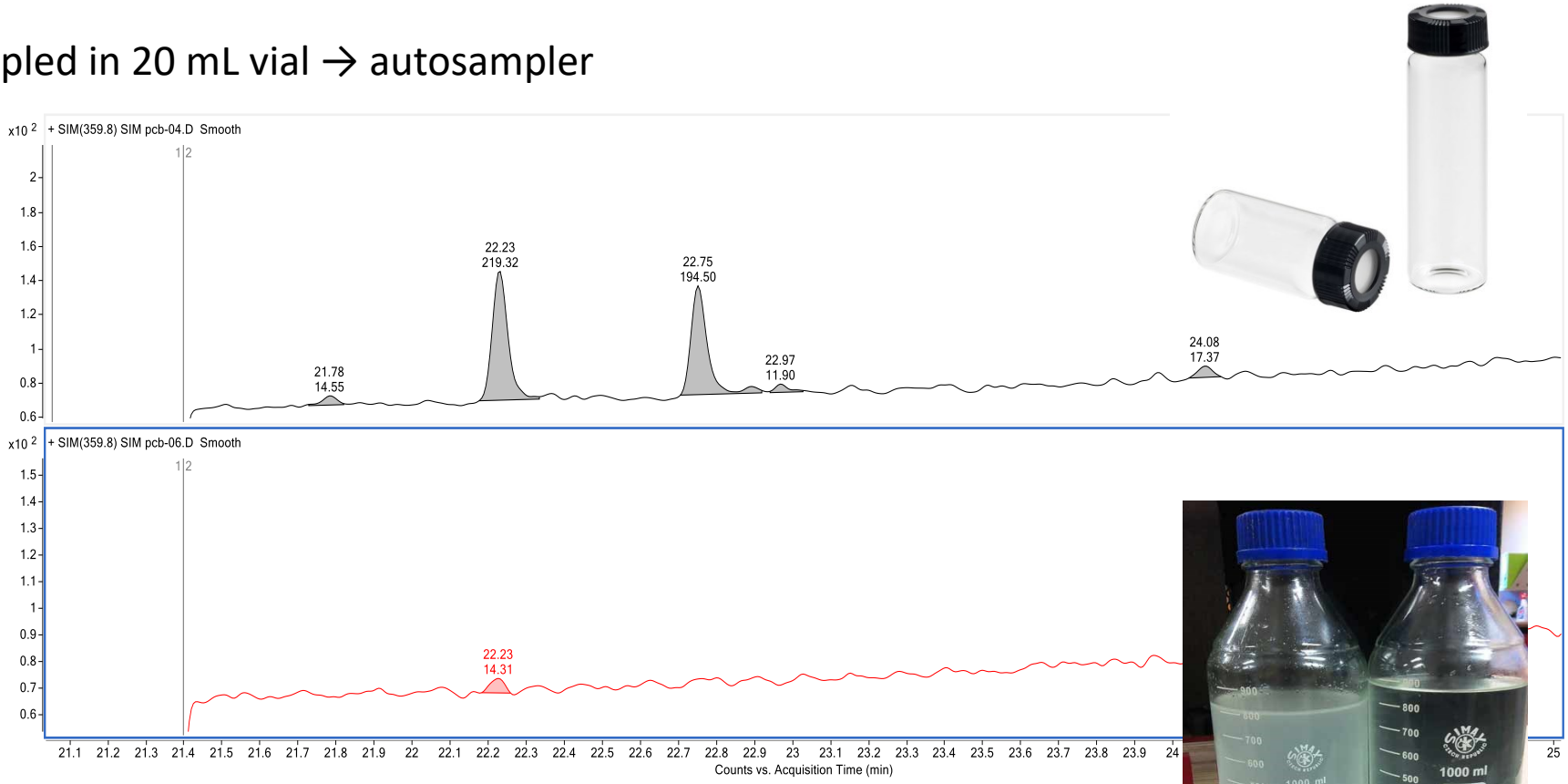
MS → MS/MS

POPs in surface water using automated µLLE - followed by LVI-PTV - GC-MS/MS



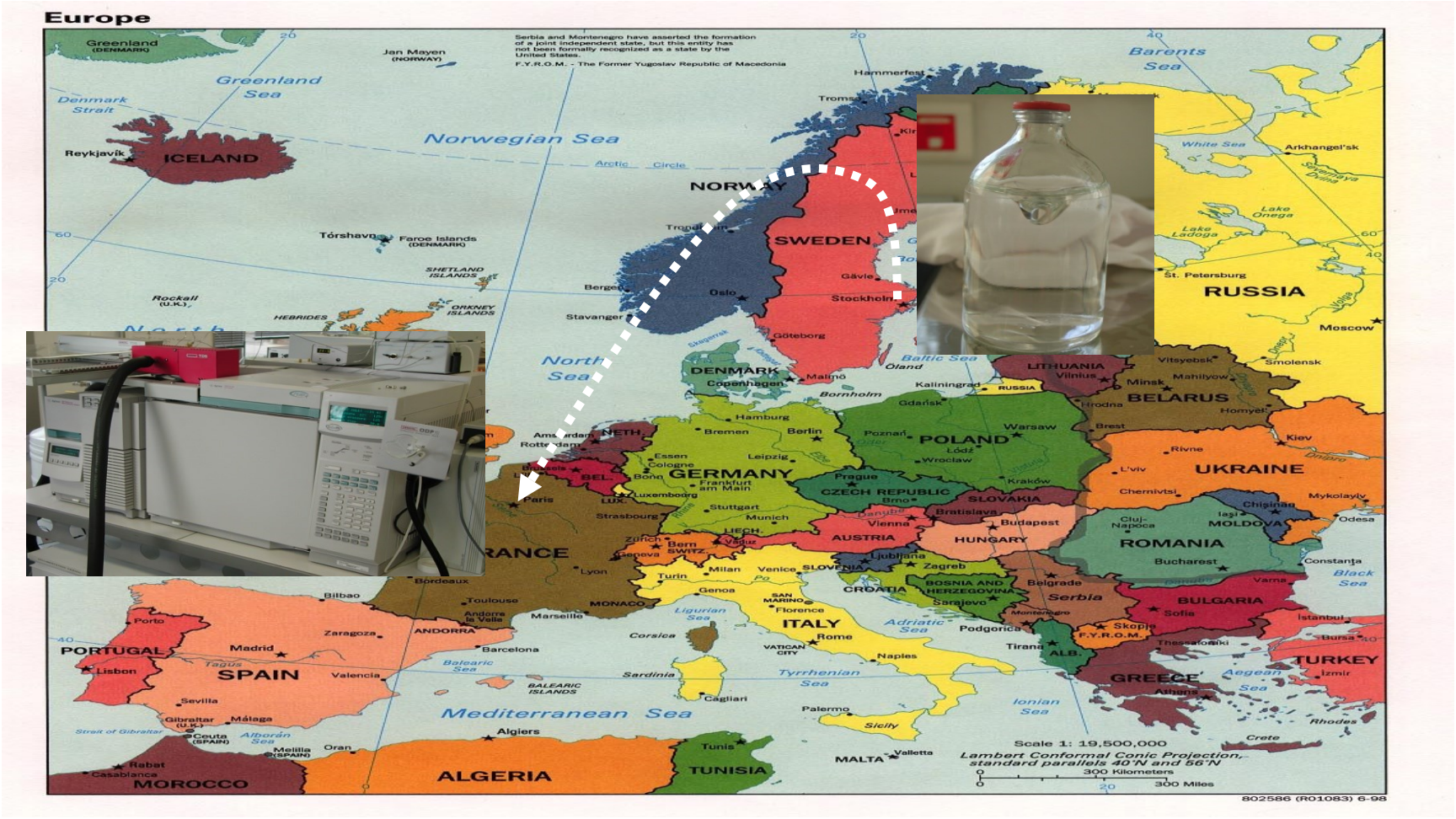
But... Sampling method should also be adapted!!!

Sampled in 20 mL vial → autosampler



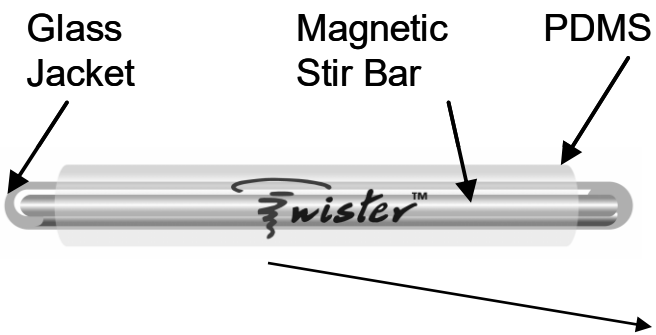
Sampled in 1 L flask → aliquot to 20 mL vial → autosampler

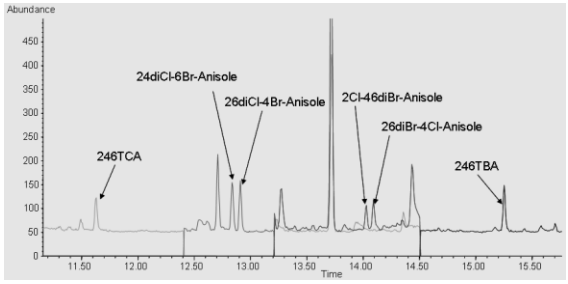
On-site SBSE TD-GC-MS



D. Benanou, presented at 27th ISCC, Riva del Garda, Italy, May 2004

On-site SBSE TD-GC-MS: Veolia ARISTOT





Method Translation

Automated determination of Hydrocarbon Oil Index (HOI) in water according to ISO 9377-2

Reference method (ISO 9377-2)	Automated
900 mL water + IS	17 mL + IS
LLE with 50 mL pentane	µLLE with 1 mL hexane
Shake	**QuickMix (15 min)**
Drying/Clean-up on Na_2SO_4/Florisil	**Decap** Transfer 600 µL to 2 mL vial with Na_2SO_4/Florisil Vortex
Concentration to 1 mL	**-**
GC-FID analysis 1 µL injection	GC-FID analysis 100 µL injection **(COC with EVE)**

Automated determination of Hydrocarbon Oil Index (HOI) in water according to ISO 9377-2

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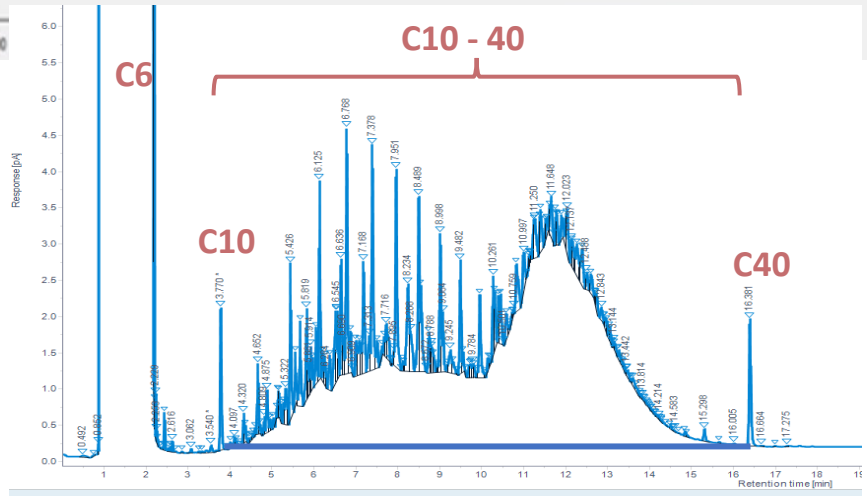
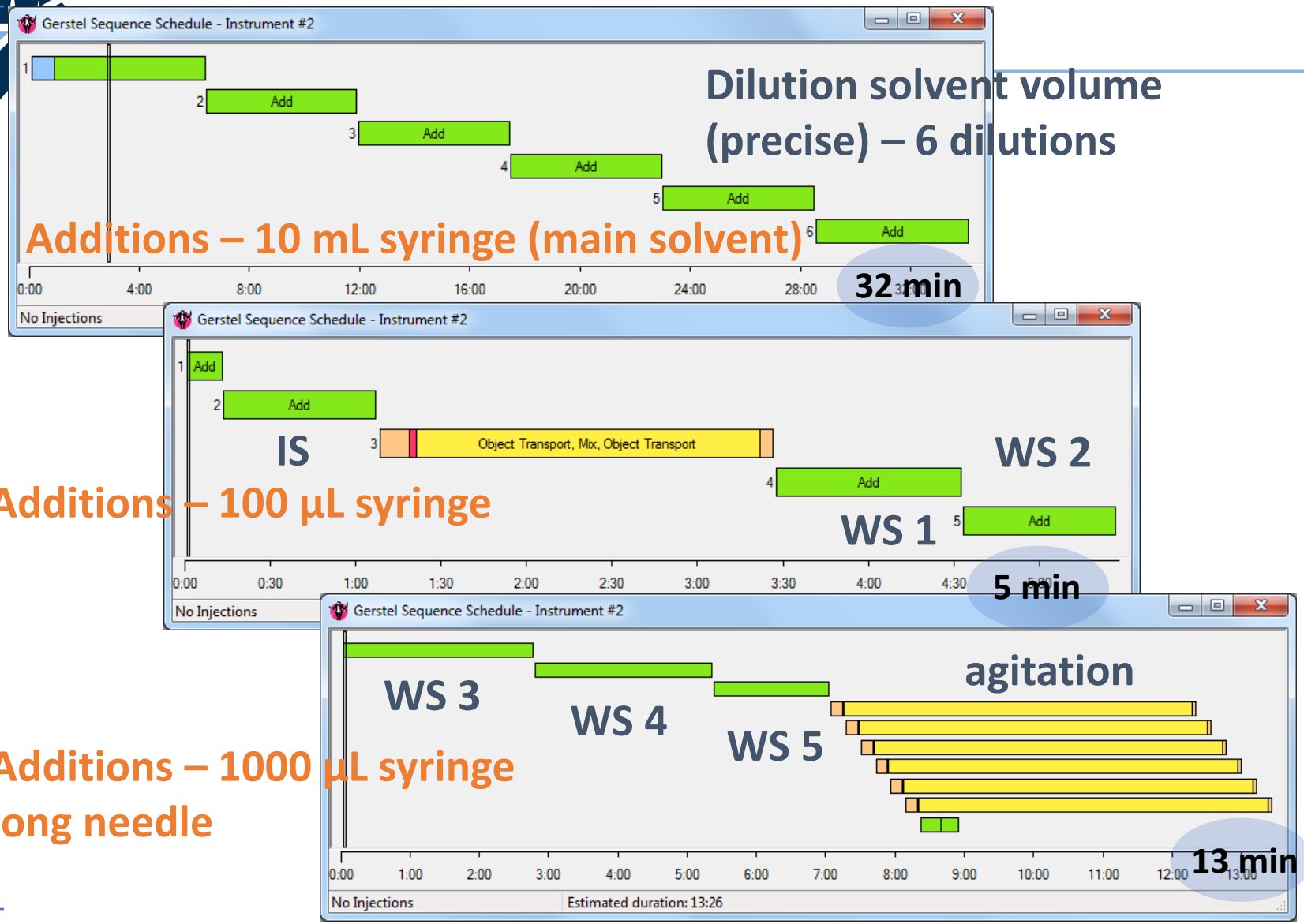


Not only for complex sample preparation methods

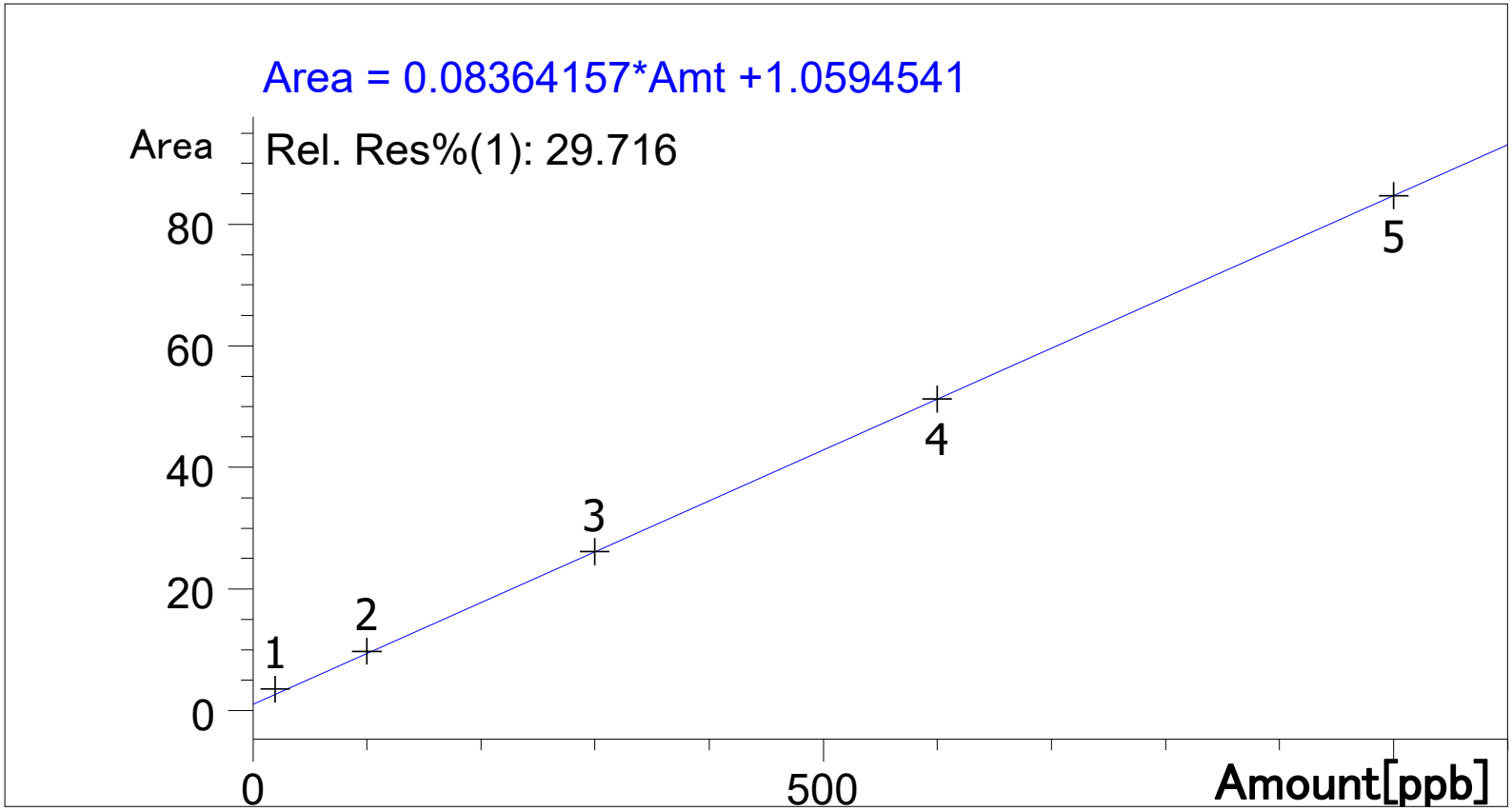
Fully Automated Dilutions

Fully Automated Dilutions

Batch sequence (ca. 50 min)



Fully Automated Dilutions

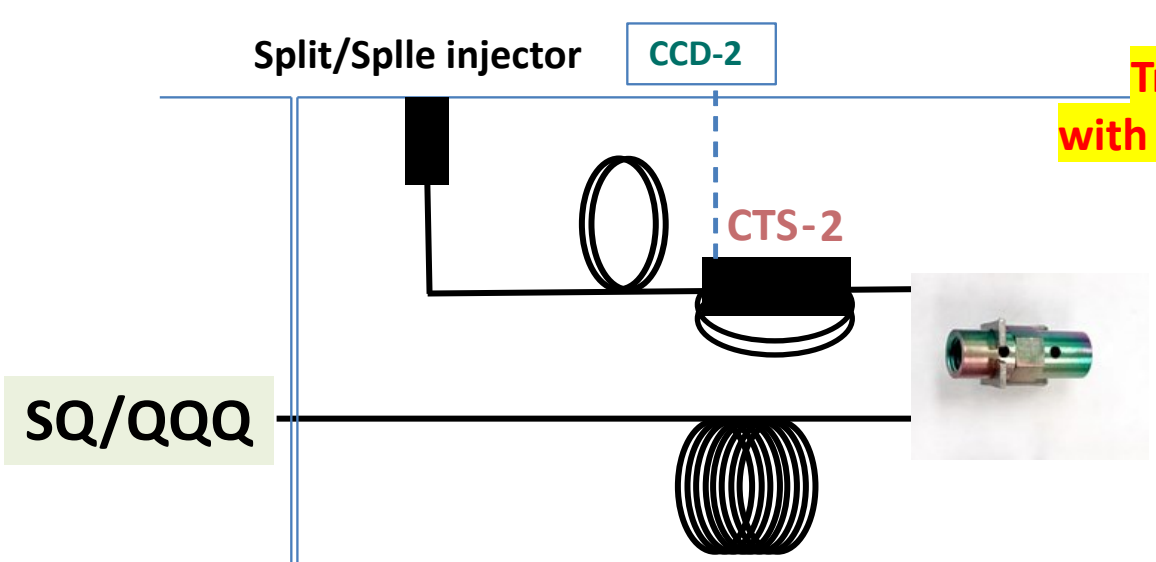


Correlation coefficient: 0.99983

NO internal standard !!

SHS – CTS-2 Solution for VOC analysis

VOCs @ low ng/L level
P&T? DHS?
Try Static Headspace Sampling with on-column focussing (liq N_2 free)



Deactivated Ultimate Union

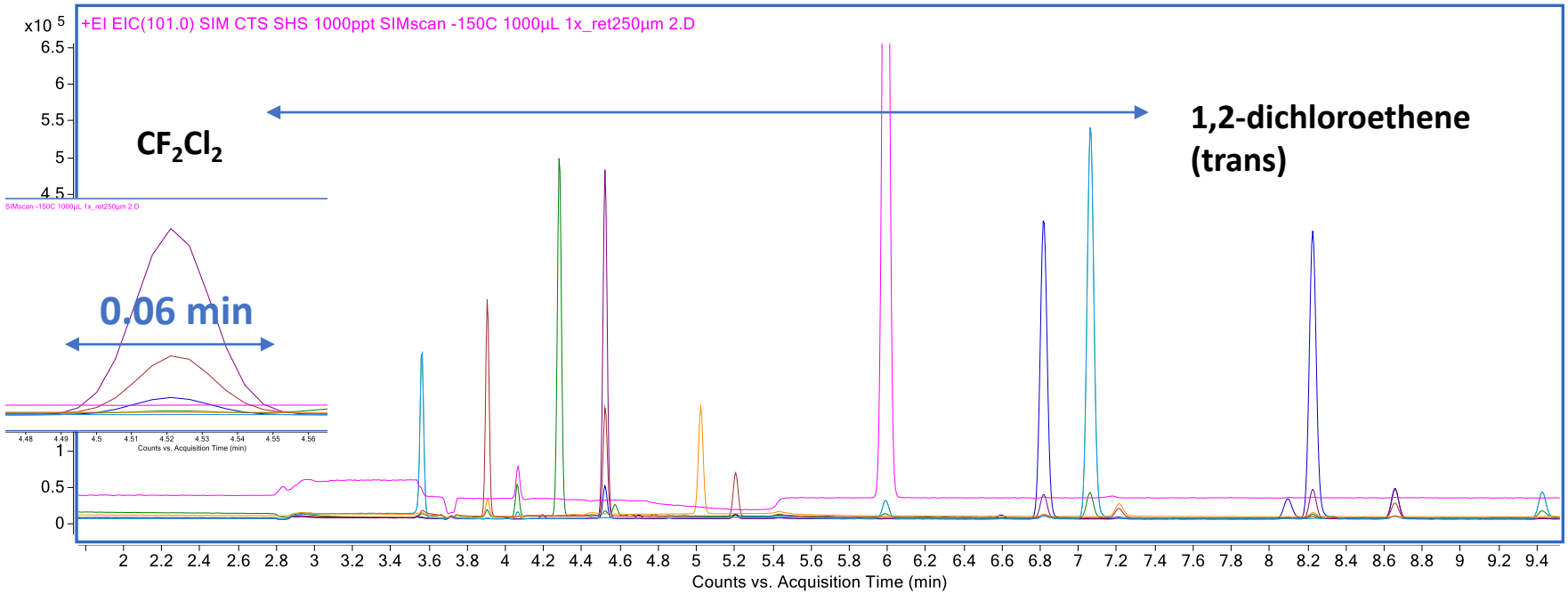
Analytical column
VOC column ((type VRX / DB-624)

Sensitivity also determined by the MS source selected, ie inert source, extractor lens ion source, High Efficiency source (HES) or by the type of MSD, i.e. SQ or QQQ.

SHS – CTS-2 Solution for VOC analysis

Multiple systems installed in Routine water/environmental labs

For example: 10 ng/L (ppt) with 10 mL sample = 100 pg / 10 mL HS = 10 pg onto the column with 1 mL HS injection (splitless): OK with HES-MSD in SIM



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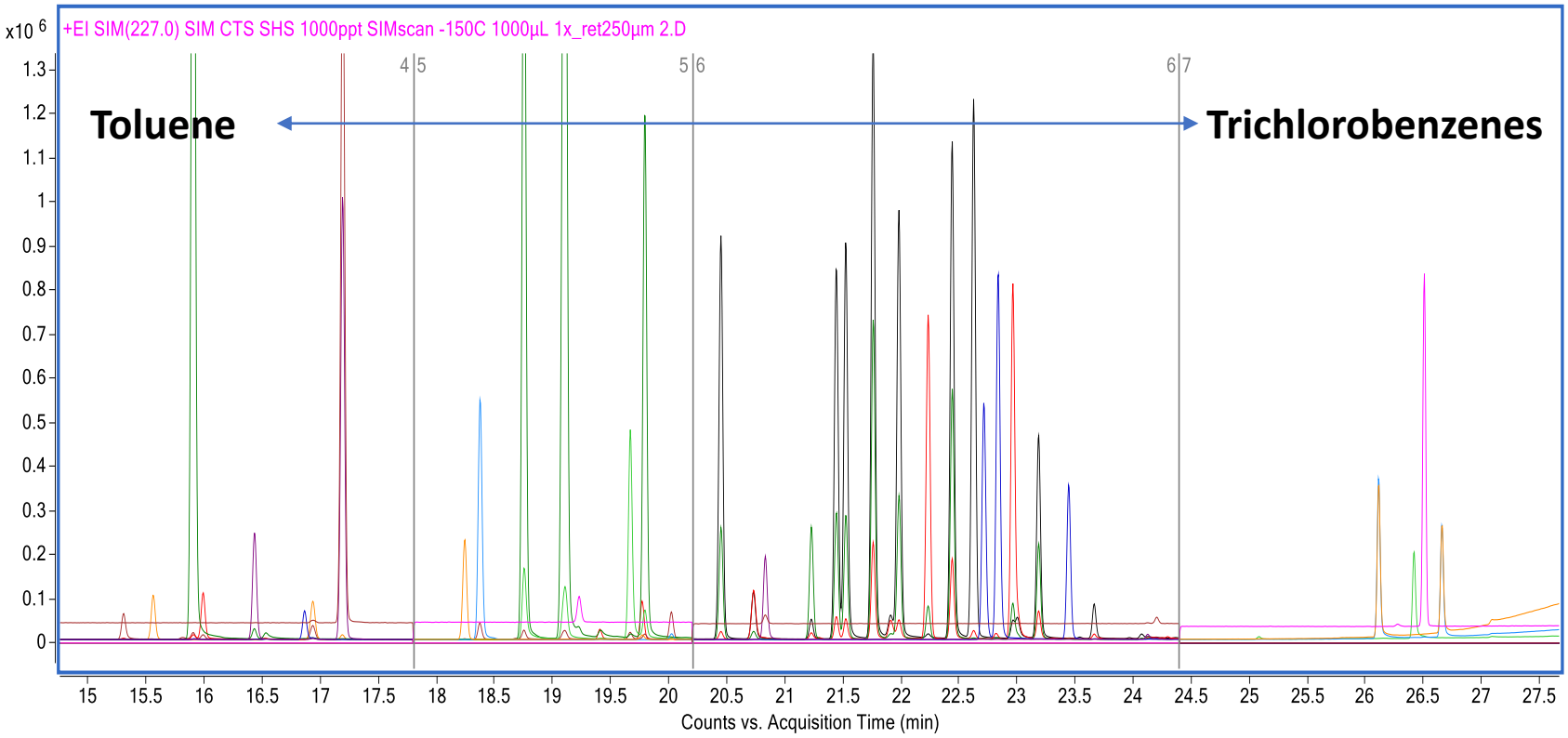


On-line or off-line?

- Important remark: On-line is not always best choice
- Productivity (Sample throughput) can be higher with off-line approach (batch processing)

Compromised data?

SBSE in Water Analysis

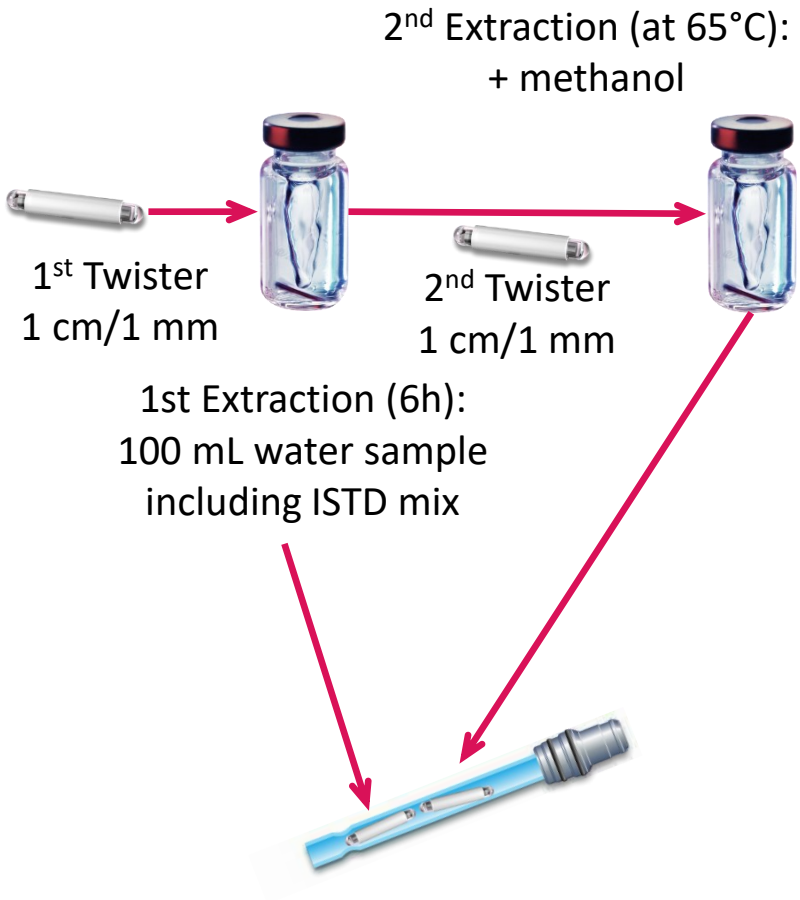
- CEN/TC 230 meeting – Paris, March 14-15, 2019
  Evaluation of analytical methodology for whole water measurements in the framework of the EU Water Framework Directive.
- SBSE based methods
  - Well documented in literature
  - Extremely sensitive (pg/L)
  - Low external contamination (phthalates)
  - Concern: Suspended Particulate Matter (SPM)
- Recent research (Gerstel)
  - Dual SBSE extraction – single shot GC-MS/MS method
  - Validated for 100 priority pollutants according to WFD
  - Matrices spiked with 50-100-150 mg/L WEPAL SETOC 745 to simulate SPM

Should we stick to “conservative” methods?

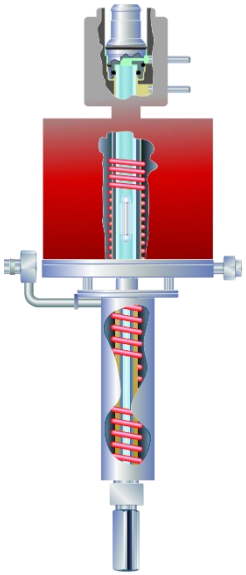
- “Official” LLE based methods are also only validated for low (< 150 mg/L) levels of SPM.
- What about (SPE)-LC-MS/MS methods?
  - Direct aqueous injection of water samples (after filtration):
    - no SPM included!
    - Ion suppression?
  - On-line SPE-LC-MS:
    - Automated
    - Highest sensitivity
    - Filter or risk of blockage?
    - Are POPs extracted on (ad)sorbent?
  - Off-line SPE:
    - Automated
    - Less sensitive: only fraction of sample injected
    - More flexible: add Vortex, filtration, concentration... **SPM extraction?**

SBSE in Water Analysis

Sample Preparation



Thermal Desorption



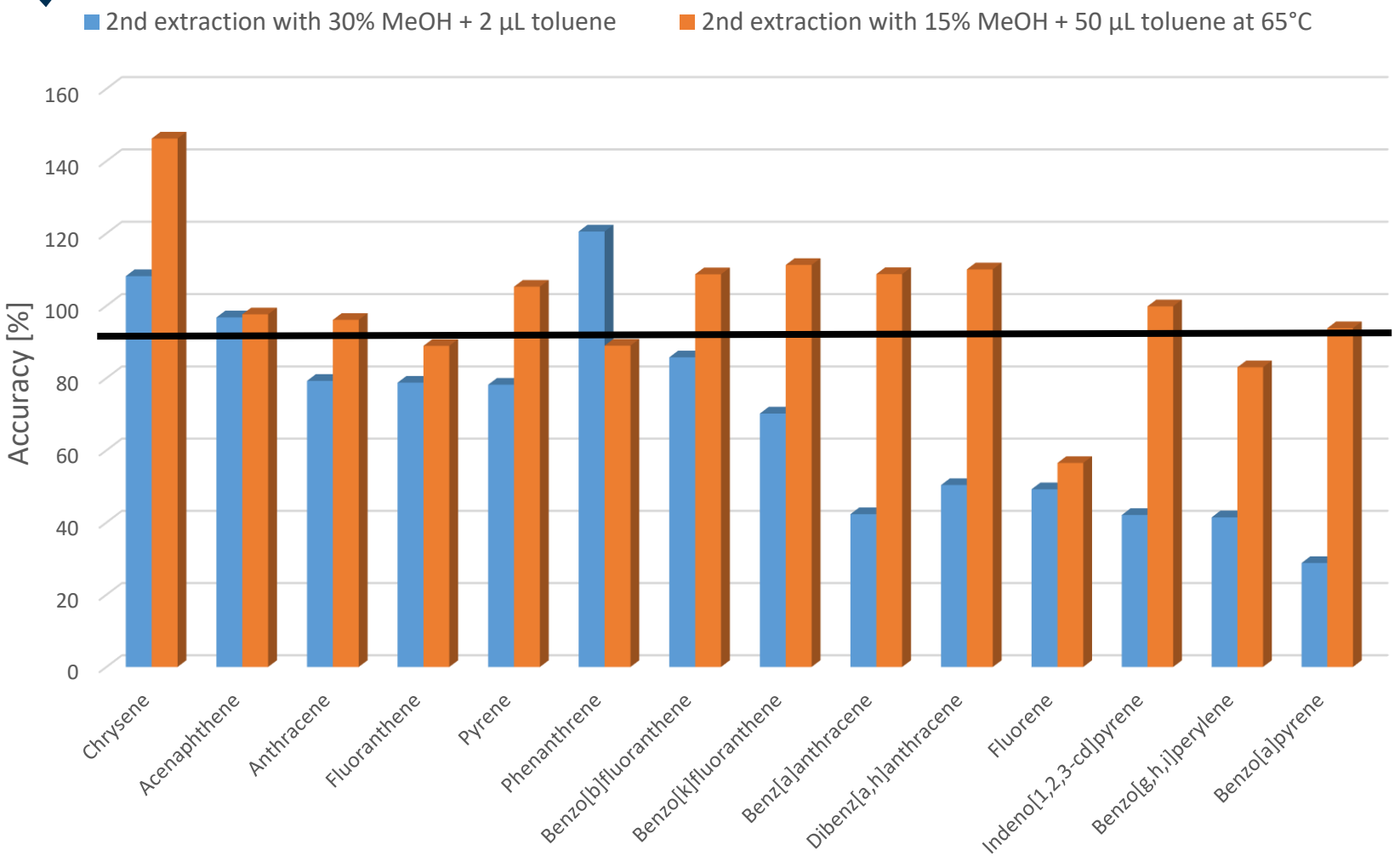
Thermal Desorption Unit
with cryofocussing in
Cooled Injection System

GC/MS/MS

7890/7010
HP-5ms UI 30m, 0.25 mm, 0.25 µm
60 °C, 1 min, 40 °C/min, 120 °C , 5 °C/min, 310 °C, 10 min
MRM

Data: O. Lerch, Gerstel GmbH

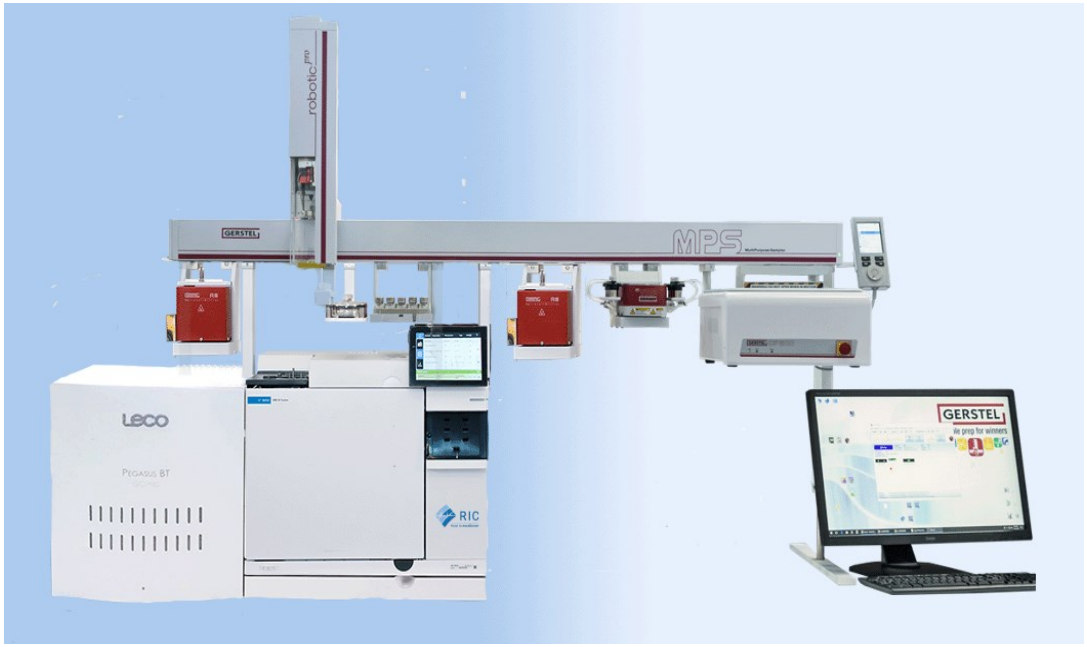
Extraction of Particle Adsorbed Analytes



Data: O. Lerch, Gerstel GmbH

Automated Fiehn metabolomics

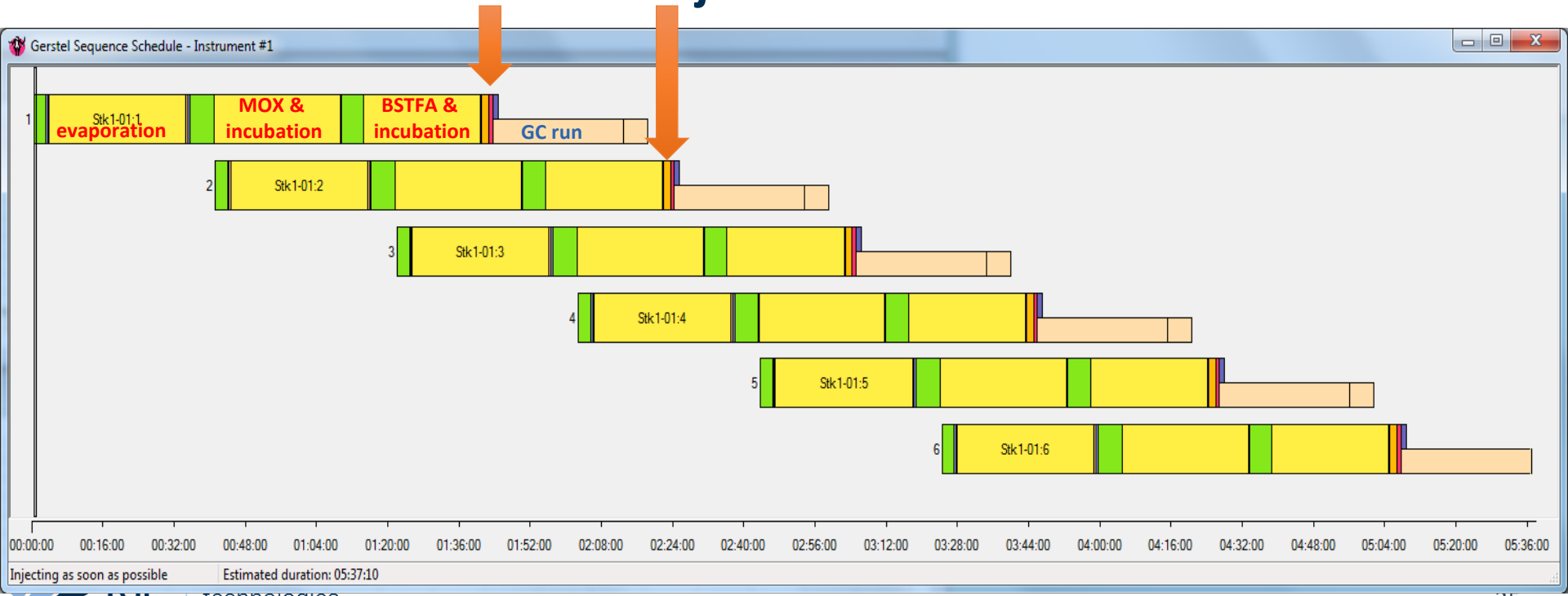
- **This system will allow you to perform the following types of analysis :**
  - **Sample preparation: just in time oximation & silylation**
  - Liquid injection
- System is upgradable to TD, DHS and other sample preparation modules



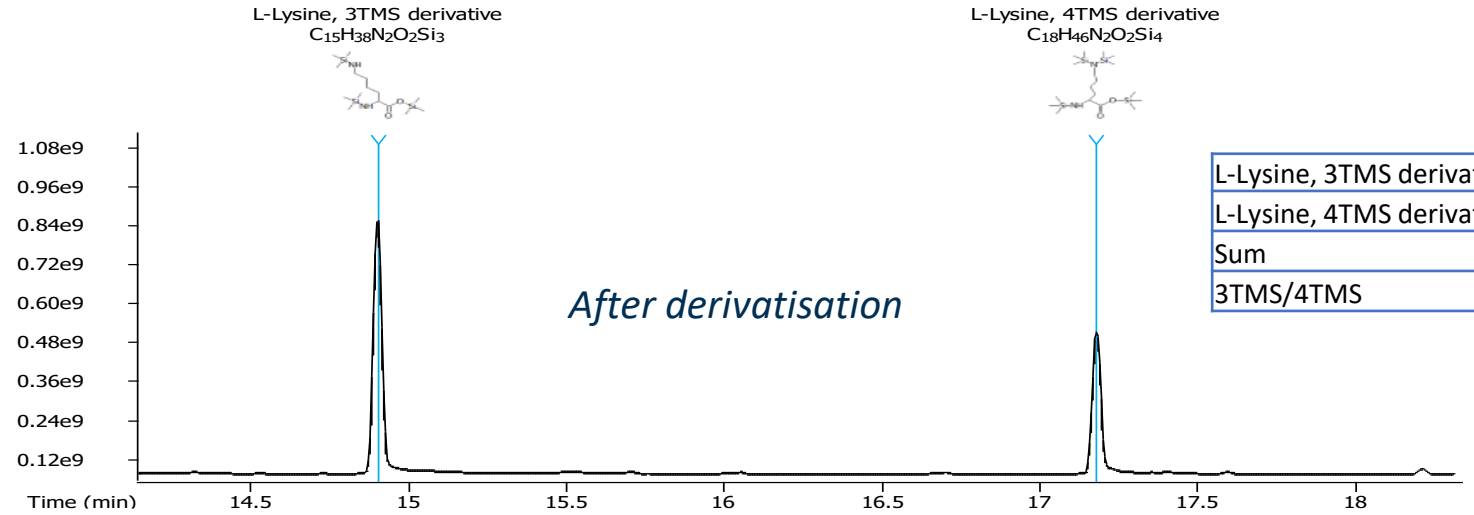
Automated “just in time” sample preparation

- The unique **PrepAhead** function of the MAESTRO software enables thermostating the samples during the GC-MS run of the previous sample, this way ensuring optimal productivity and utilization of the GC-MS system

“Just in time” injection

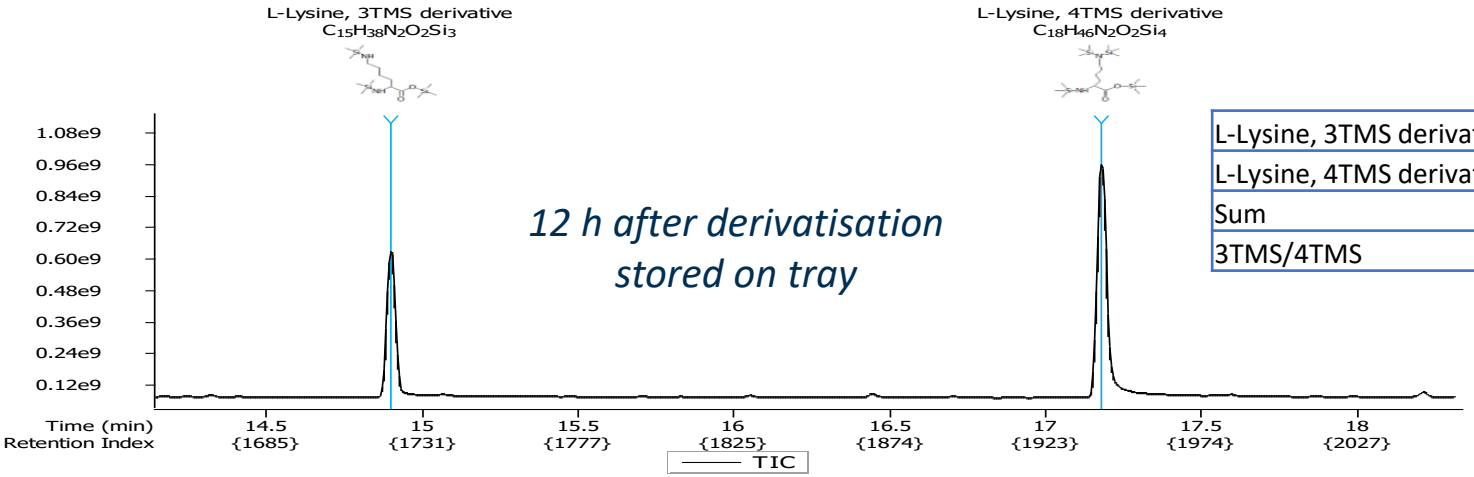


Automated “just in time” sample preparation



After derivatisation

L-Lysine, 3TMS derivative	1792362372
L-Lysine, 4TMS derivative	739498970
Sum	2531861342
3TMS/4TMS	2.42



12 h after derivatisation stored on tray

L-Lysine, 3TMS derivative	1239928358
L-Lysine, 4TMS derivative	1717200378
Sum	2957128736
3TMS/4TMS	0.72

Flexibility - Multi-methods – Safety & Health

Automated Sample Preparation in Lipid Analysis

- Biological samples:
  - Small volumes – all in 2 mL vials → Agilent 7693 ALS (dual)
- Fats, oils, fatty foods (nuts)
  - Extraction
  - Saponification
  - Derivatization
  - LLE + centrifugation
  - concentration

"Fame-analyzer" ????

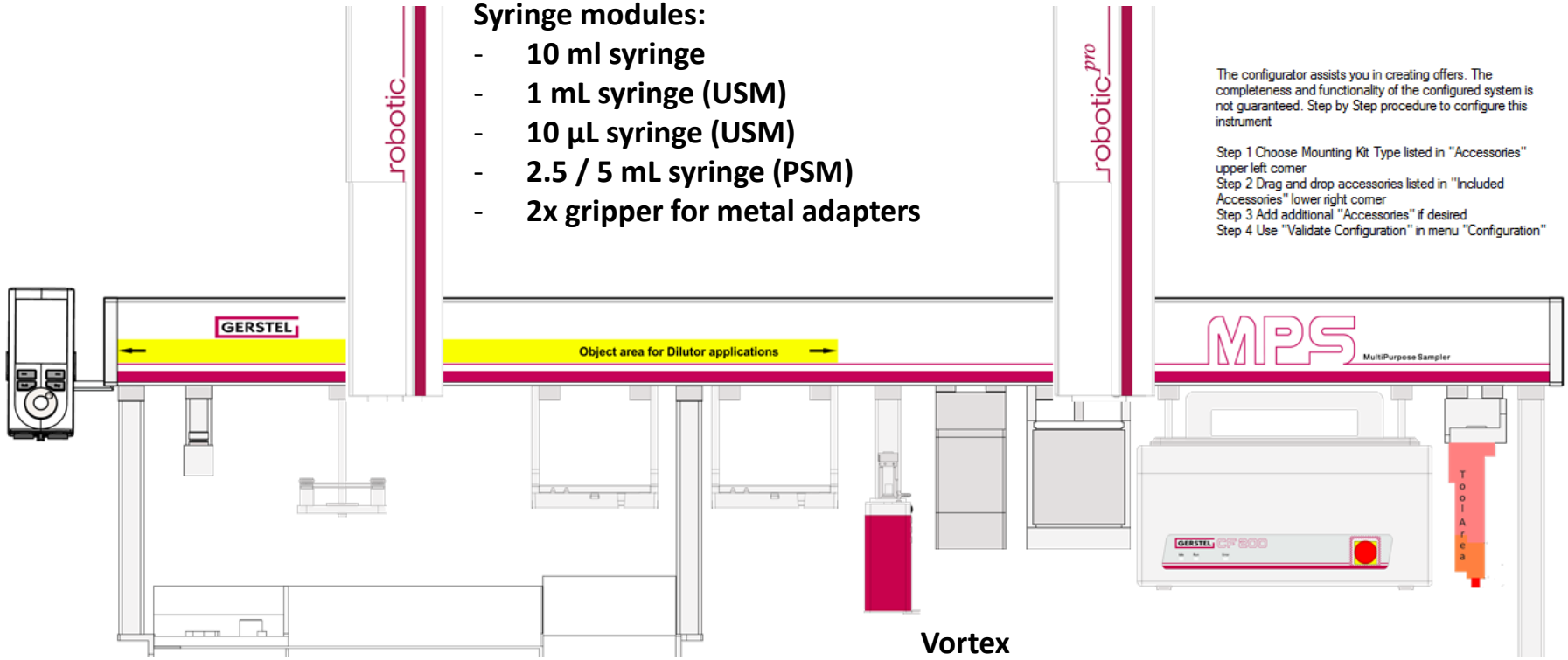
FAILED

Flexible sample prep robot

Example: configuration for 3 FAME methods

Syringe modules:

- **10 ml syringe**
- **1 mL syringe (USM)**
- **10 µL syringe (USM)**
- **2.5 / 5 mL syringe (PSM)**
- **2x gripper for metal adapters**



Vortex

CF200 centrifuge

Large wash
3 x 180 mL

Tray
144 x 2 mL

Tray
60 x 10/20 mL

SFS 3

Agitator

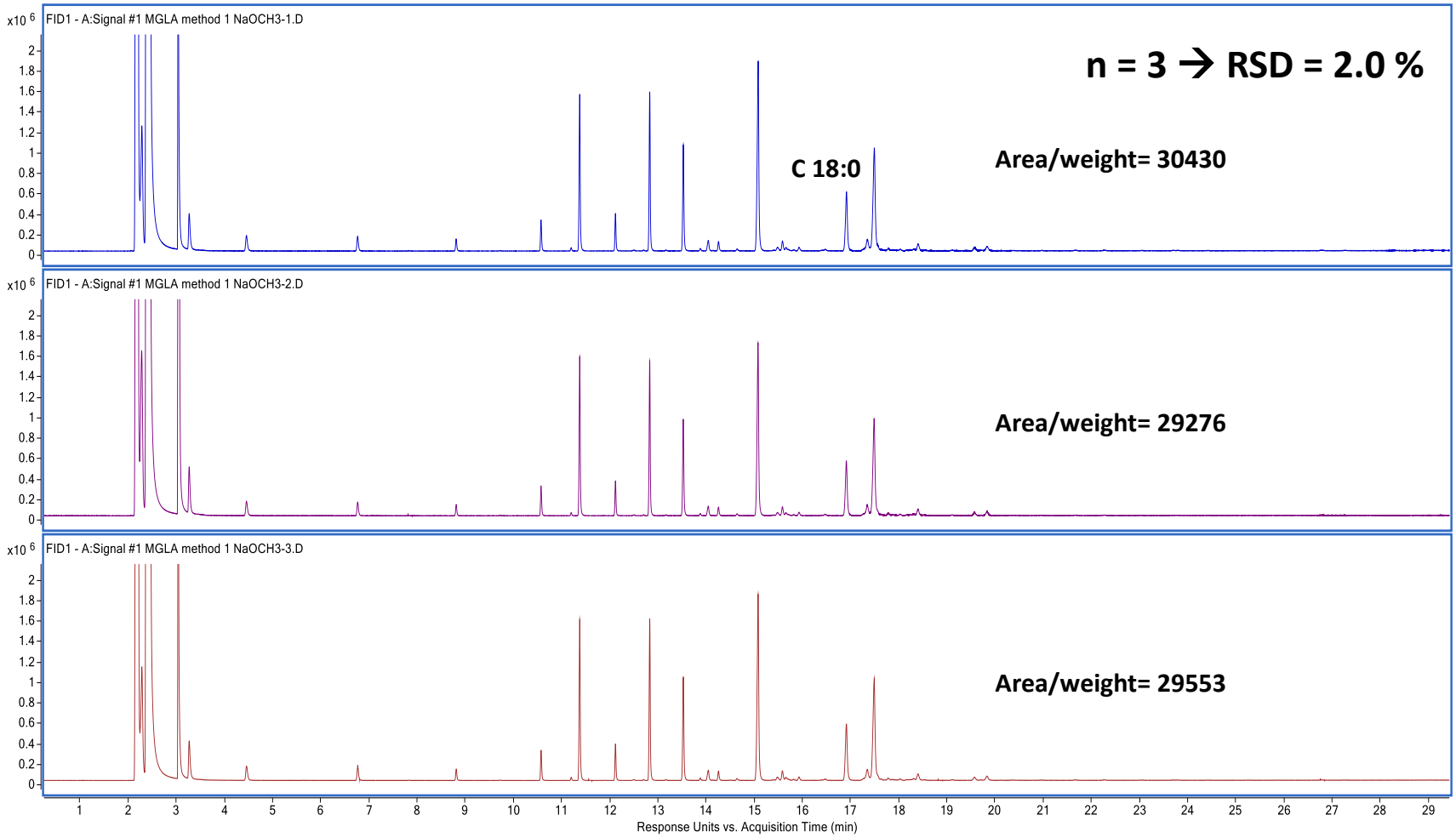
Sample prep system for 3 “FAME” methods

1. ISO 12966-2: animal & vegetable fats & oils, no milk
   → KOH/MeOH transesterification

2. ISO 12966-4: crude & refined hydrogenated animal & vegetal fats & oils – no milk/diary (or conjugated linoleic acid)
   → BF_3/MeOH

3. ISO 16958: milk, infant food (long chain PUFA)
   → Sodium Methoxide ($NaOCH_3$)
   (no fat extraction, direct transesterification in food)

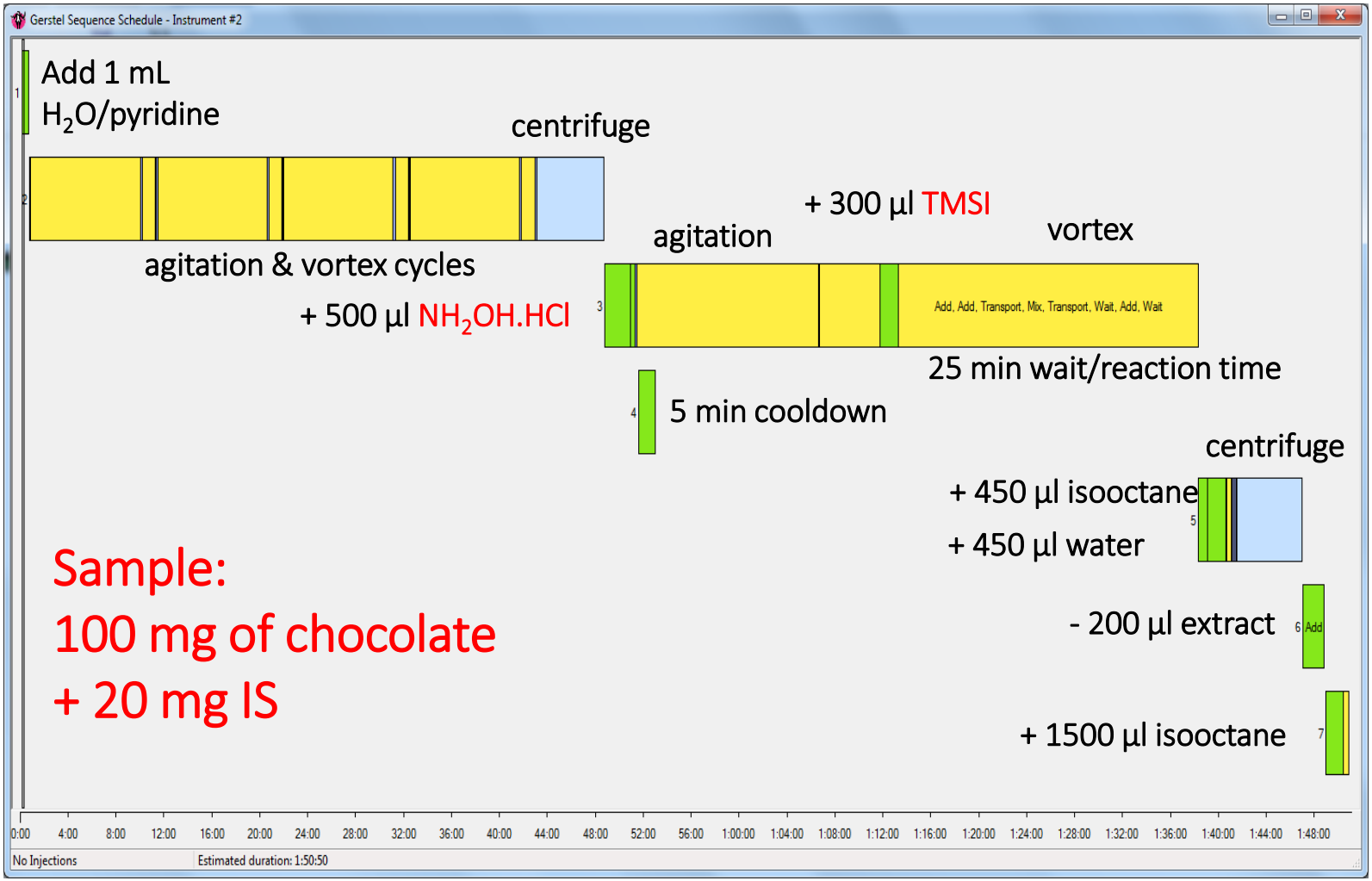
FAME analysis fat sample – Method 3: $NaOCH_3$



FAMES analyzer – Area % – BCR 162R (Soya / Maize oil blend)

% FAME	Certificate	BF_3	KOH	$NaOCH_3$
C16:0	10.74	10.8	10.93	11.01
C18:0	2.82	2.78	2.78	2.81
C18:1c	25.4	24.91	25.08	24.97
C18:2c	54.13	54.73	54.45	54.48
C18:3 n3	3.35	3.21	3.20	3.18

Same system: analysis of sugars in chocolate



Installation in Lab

Conclusions and call of duty

- Take advantage of state-of-the-art GC and MS
- Miniaturized – automated – reduced solvent consumption – increased productivity – safer
- But…
  - Sampling must be reconsidered in function of (automated) sample prep
  - New methods should be compared to existing ones and properly validated (certified materials, proficiency tests, demonstrate equivalence)
  - Take into consideration:
    - Do a method translation exercise before you start
    - On-line or off-line
    - Multiple methods on same system

Acknowledgements

- Our colleagues at RIC Technologies & RIC Resolve
- Agilent Technologies, Gerstel GmbH & Co. KG & Leco
- Our customers

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