Improve your Laboratory Productivity by Automating & Miniaturizing Sample Preparation for GCMS & LCMS Analysis

26MAR 2021

Webinaire

CECM

About RIC group

- Privately-owned centre of excellence in the field of analytical solutions

- The Research Institute for Chromatography (RIC) was founded by Prof. Pat Sandra in 1986, now headed by Tom & Koen Sandra

- Main office & labs based in Belgium (Kortrijk)

- Sales office in Lyon

- Supporting customers in various industries with scientific know-how & technical expertise for almost 35 years

About RIC group

RIC | technologies

RIC | resolve

RIC | biologics

RIC | omics

RIC technologies

Analytical equipment and state-of-the-art instrumental solutions to expand your in-house capabilities.

RIC resolve

Chromatography services for consumer products, food, environmental and petrochemical industry.

RIC biologics

All-round analytical services for pharma & biotech working on biological products and chemical entities.

RIC omics

Understanding biological pathways for biomarker and drug target discovery.

RIC | group

21ˢᵗ Century Sample Preparation

- Since many years keywords in sample prep are:
  - Automation
  - Miniaturization
  - Solventless ("green")
  - High productivity:
    - Samples/day
    - Cost/sample
    - Safety

- Instrumentation: orders of magnitude increase in sensitivity using state-of-the-art MS (MS/MS, TOF…)

- Some examples…

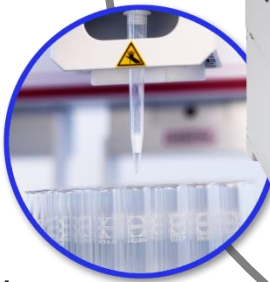Dynamic Headspace DHS

TDU Thermal desorption and PYROlysis

Solid Phase Extraction SPE

ATEX Thermal Extraction in µVials

Disposable Pipette Extraction DPX

SPME & Multi-Fiber EXchange MFX

Headspace

ALEX Automated Liner EXchange

The Best Sample Preparation is …

… No Sample Preparation

- **Direct liquid injection of extracts**
  - Plant extracts: presence of non-volatile material
    - Example: atranol, chloroatranol in moss extracts
    - Observation: contamination & ghost peaks
  - Pesticides in Food
    - **QuEChERS: Quick, easy…but above all: dirty**
- **Solution: automated liner exchange (Gerstel ALEX)**

Direct liquid injection of extracts: PTV with ALEX

Septum

Adapter

Liner

Packing material (PDMS foam)
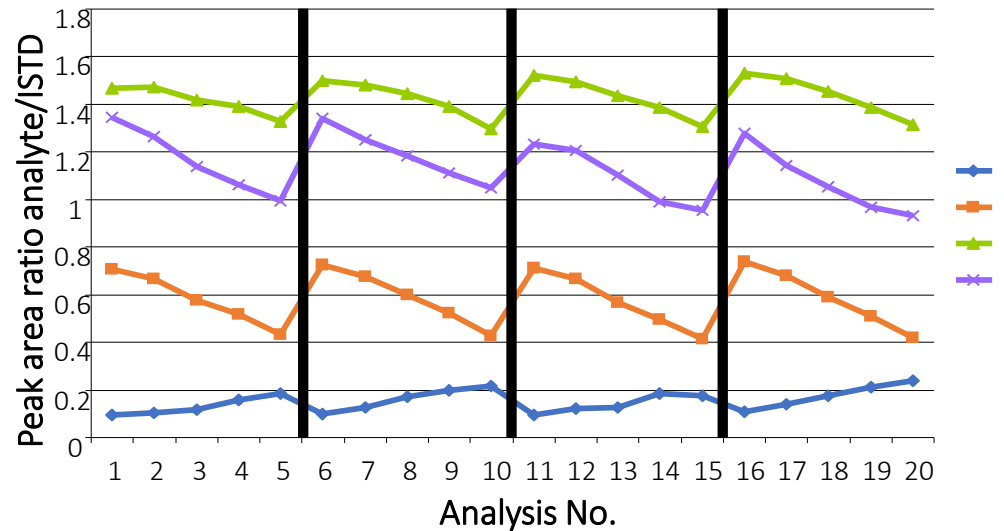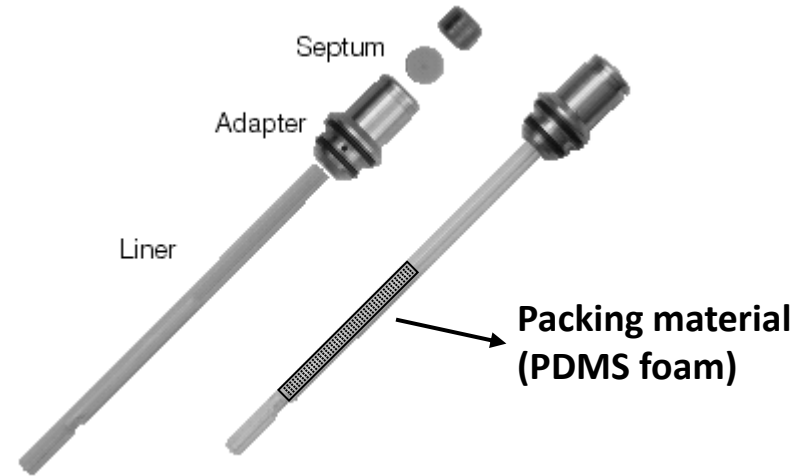
Direct liquid injection of extracts & thermal extraction: TDU - PTV

Liquid injection of extract directly into a TDU insert

**Thermal extraction (TE)**

Analysis…

TDU insert → single use

Inlet liner /column / detector protected

Miniaturization example: Trace analysis of POPs in water using µLLE followed by LVI-PTV injection and 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 = 1 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
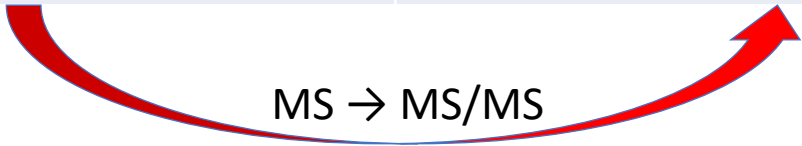
Autosampler configuration: lab-on-a-rail

Flexible configuration
Module selection according to needs
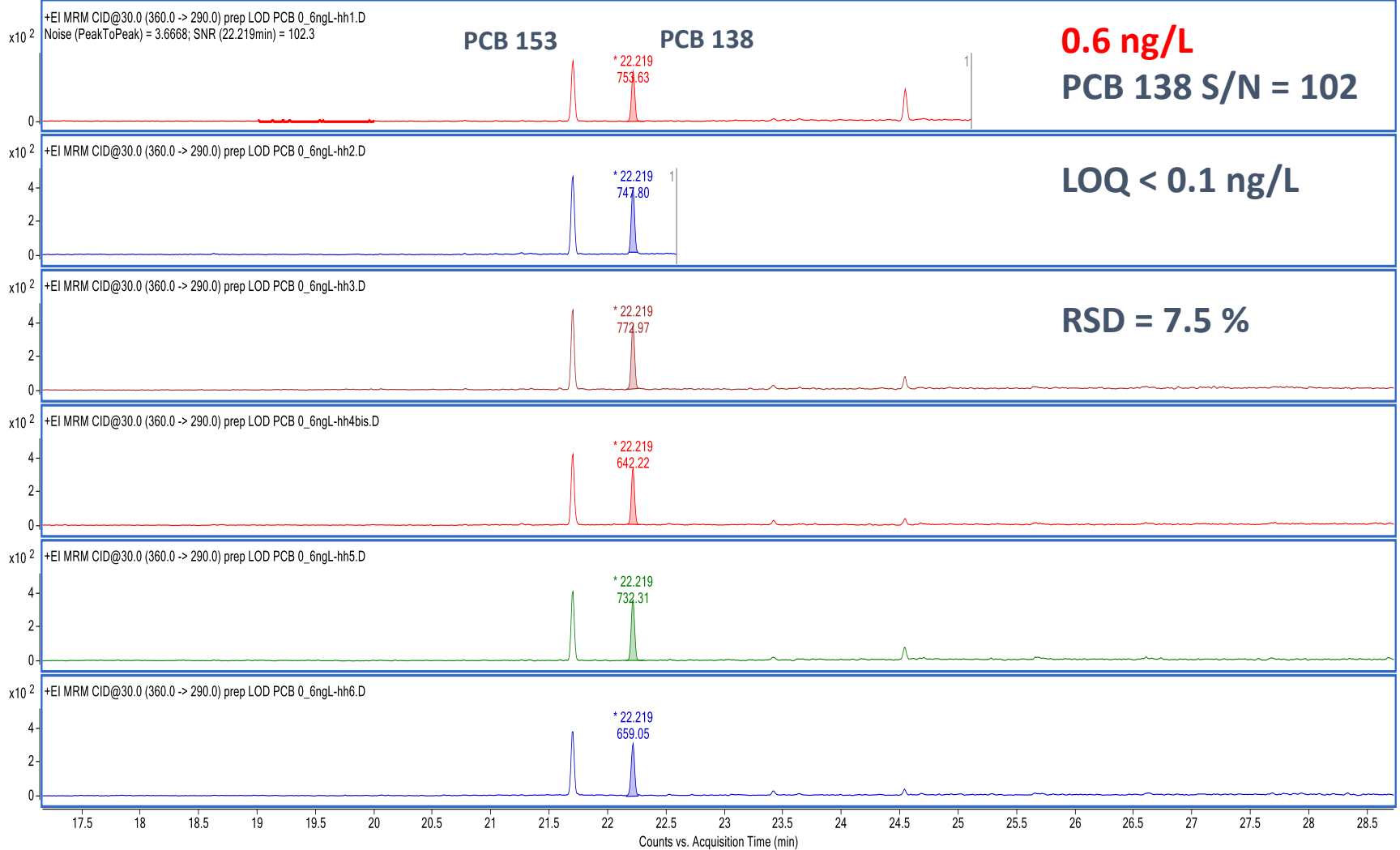Single or multiple application ?
Software control
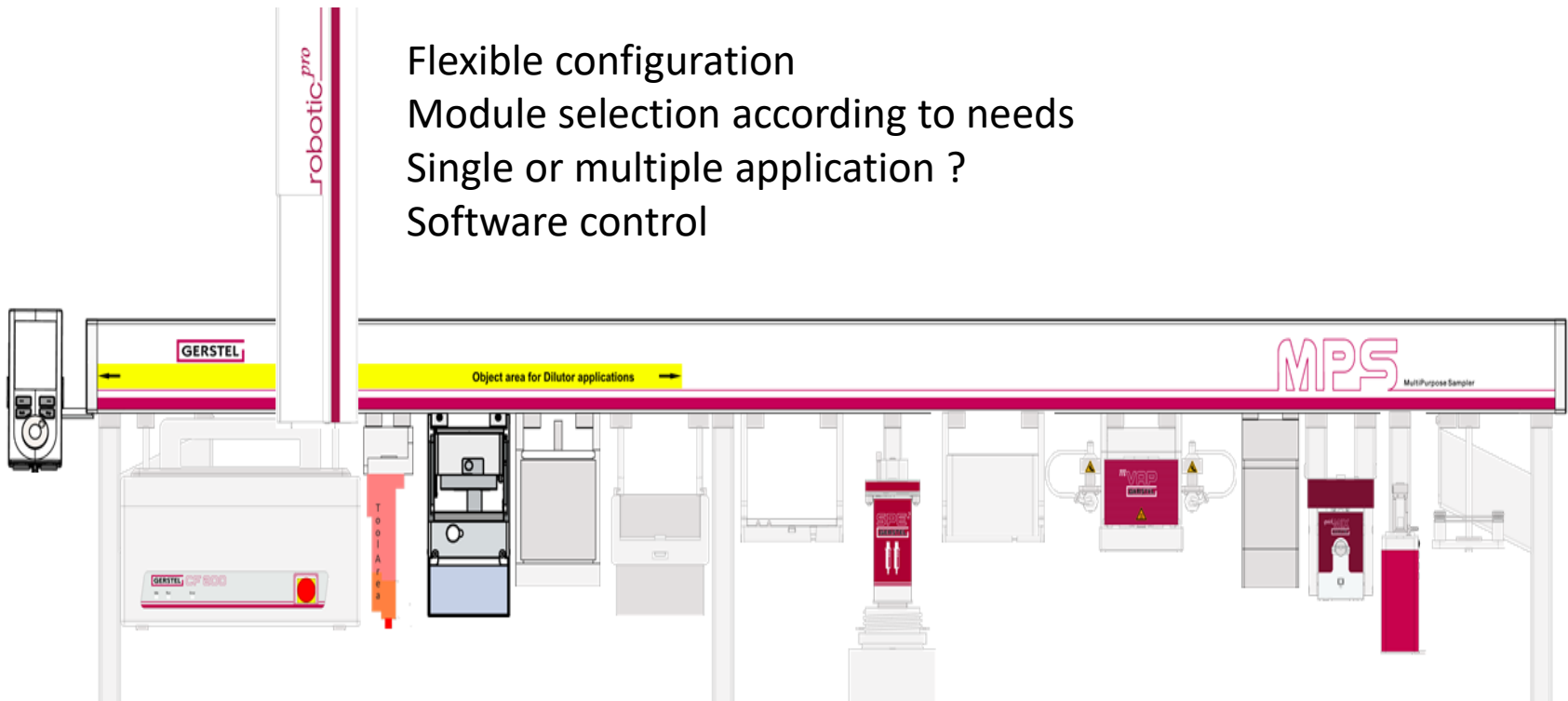
Maestro software (scheduler) – sequence
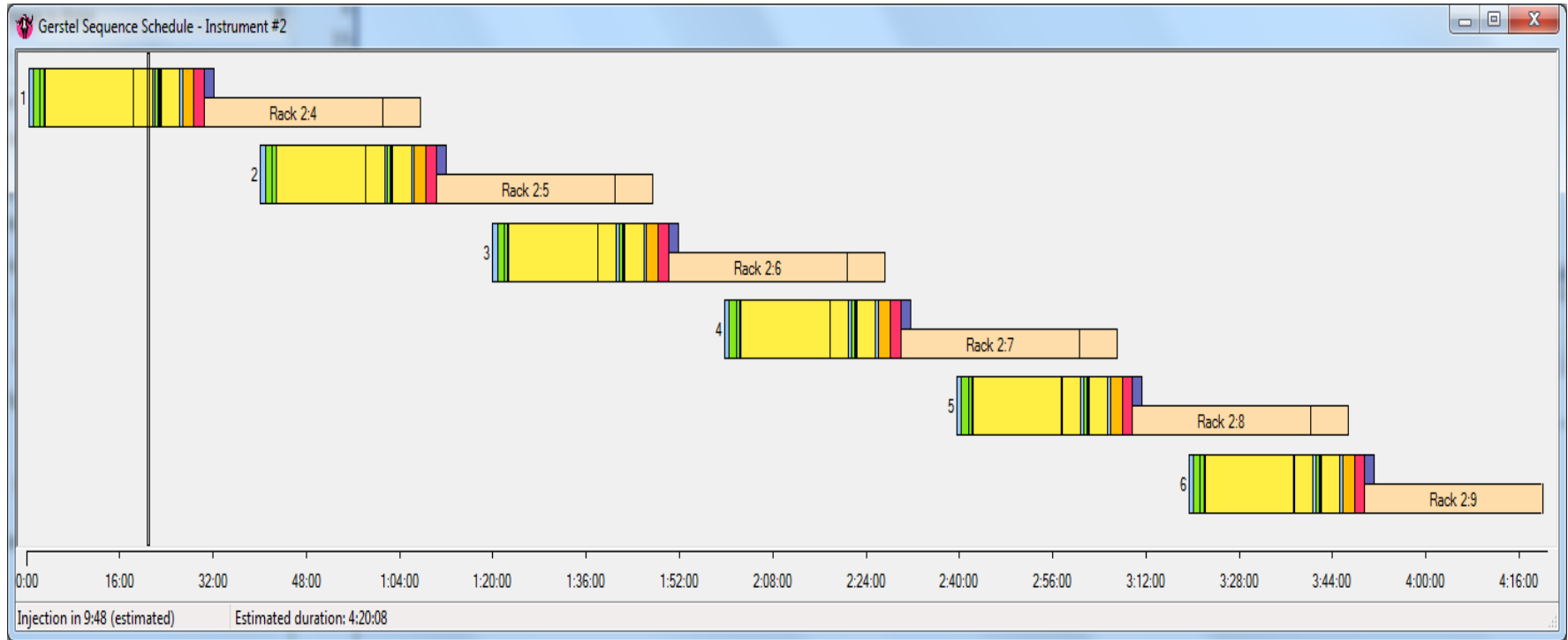
- Overlap of sample preparation & analysis
- Just-in-time sample preparation
- Calibration included

μLLE + LVI PTV GC-MS/MS – Repeatability & LOQ

0.6 ng/L
PCB 138 S/N = 102
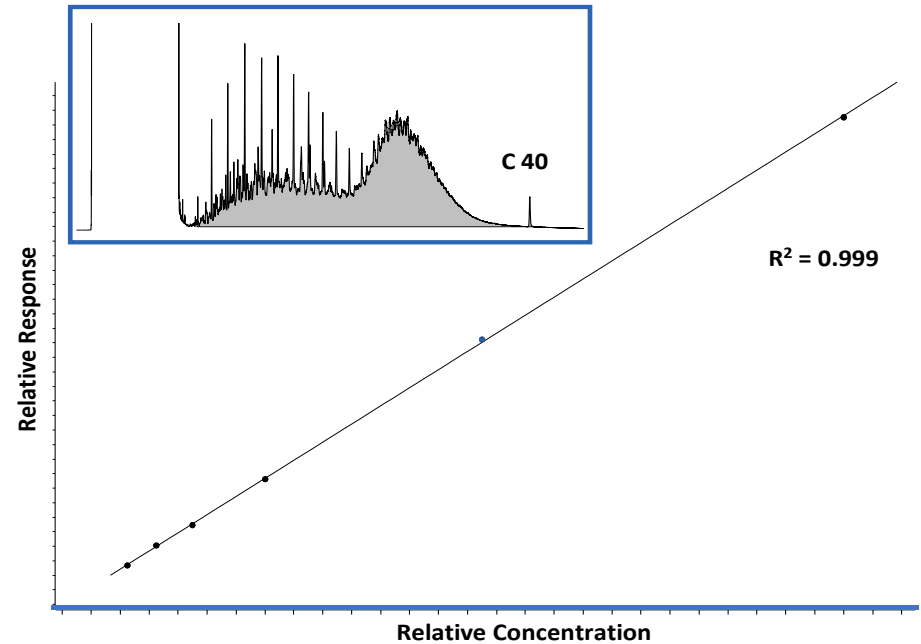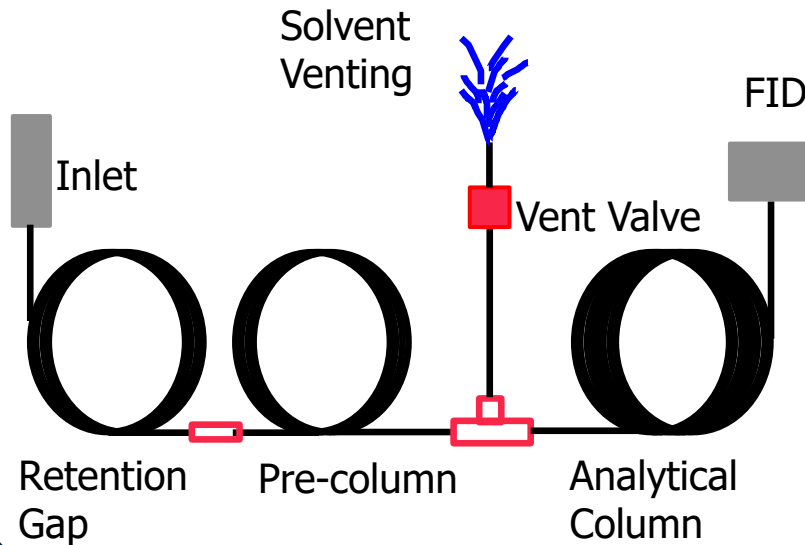
LOQ < 0.1 ng/L

RSD = 7.5 %

Miniaturization & automation of mineral oil analysis (HOI, TPH) – based on ISO 9377

- In-vial extraction
- In vial clean-up (Florisil)
- COC-based LVI on new interface

C 40

$R^2 = 0.999$

Relative Response

Relative Concentration

Solvent Venting

FID

Inlet

Vent Valve

Retention Gap

Pre-column

Analytical Column

Extension: MOSH/MOAH analyzer

- LC clean-up & fractionation of MOSH/MOAH fractions
- Injection of each fraction using COC based inlet (Grob-type LCGC interface)
- Dual FID configuration
- Dedicated software
- Optional: automated epoxidation for removal of alkenes (squalene)

Solventless extraction: SBSE for Aroma profiling

- Classical methods:
  - LLE (SDE, SAFE)
- SBSE (Twister):
  - ° 1999
  - Solvent free
  - Very high enrichment factor
  - Robust & high loadability
  - More sensitive than SPME
- Thermal desorption
  - Solventless
  - Full transfer
- GC-TOF MS
  - Untargeted analysis ("scan")
  - High sensitivity
  - Powerful deconvolution

SBSE – TD – GC-TOF MS analysis of lager beer
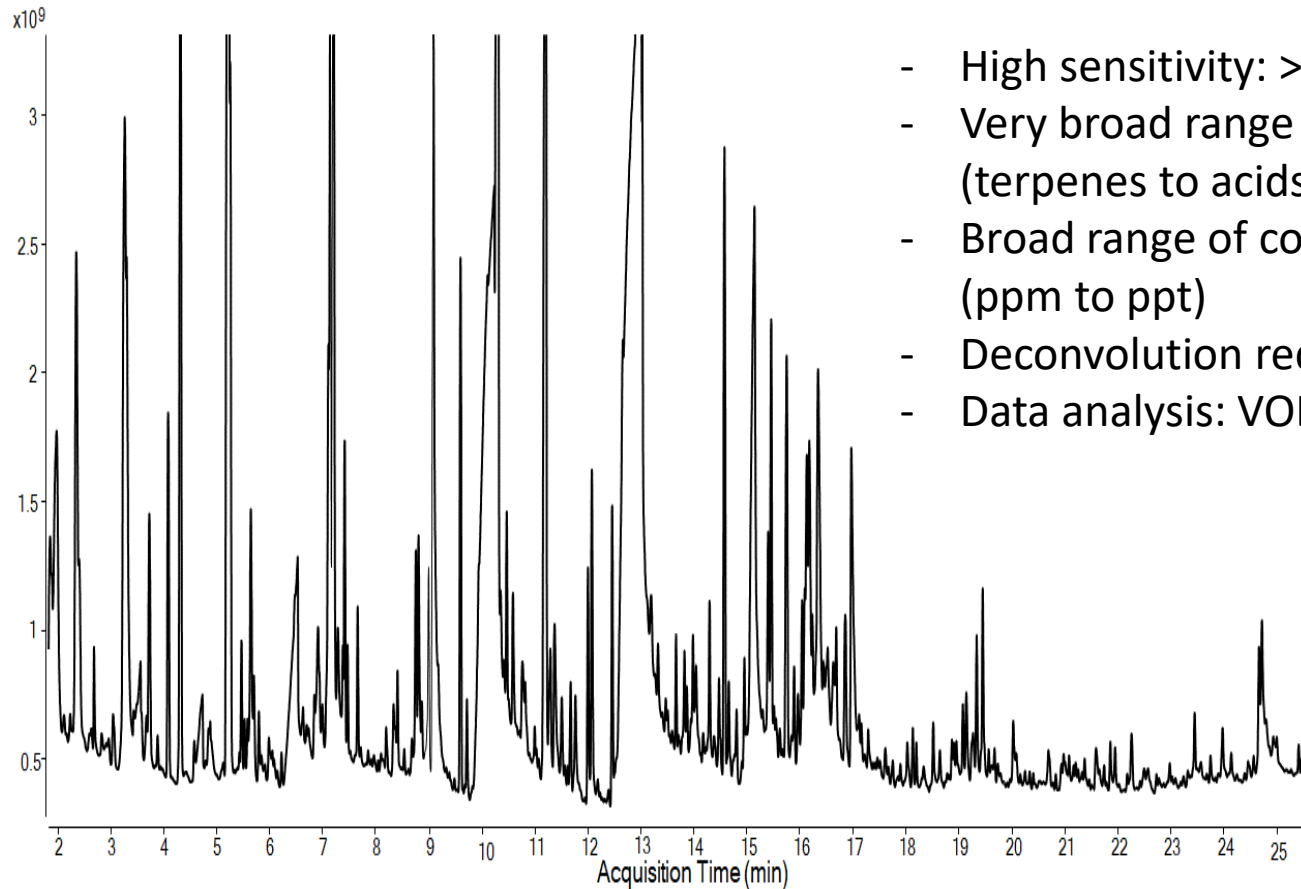
- High sensitivity: > 400 solutes detected
- Very broad range of solutes (terpenes to acids)
- Broad range of concentrations (ppm to ppt)
- Deconvolution required
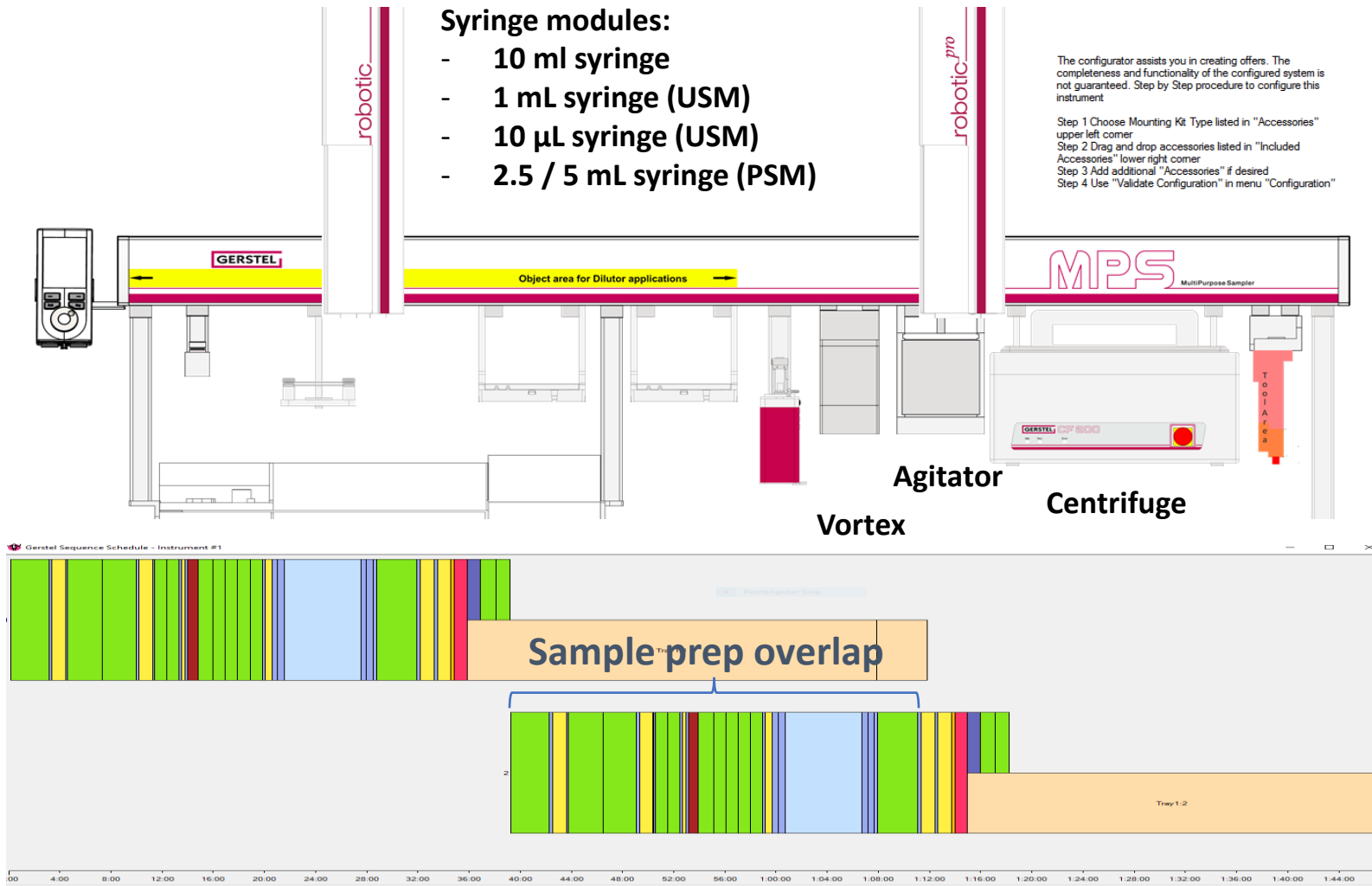- Data analysis: VOLATILOMICS

Automated Sample Preparation in Lipid Analysis

- Biological samples:
  - Small volumes
  - Homogeneous (blood, serum)
  - Sample preparation in 2 mL vials

- Food samples
  - Various matrices: fats, oils, fatty foods (nuts), diary products
  - Large sample size (homogeneity?)
  - Sample preparation includes:
    - Extraction
    - Saponification
    - Derivatization
    - LLE + centrifugation
    - concentration

**Flexible
sample prep robot**

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FAME analysis in Food: multiple methods used → customer configuration for 3 FAME methods

Syringe modules:
- **10 ml syringe**
- **1 mL syringe (USM)**
- **10 µL syringe (USM)**
- **2.5 / 5 mL syringe (PSM)**

Vortex

Agitator

Centrifuge

Sample prep overlap

Example: ISO 16958 (NaOCH$_3$ method)

- **50 mg fat or 250 mg milk powder in 20 mL vial**
- **+ 4 ml ISTD solution in MTBE**
- **Vortex**
- **+ 4 mL NaOCH$_3$ (5 % in MeOH)**
  → Start reaction
- **Vortex**
- **WAIT**
- **+ 2 mL hexane**
  (180s after "start reaction")
- **+ 8 mL buffer**
- **Vortex & Centrifuge**
- **Transfer 1 mL to 2 mL vial with CaCl$_2$**
- **Vortex (+wait)**
- **Inject 1 µL**

RSD = 2.0 %
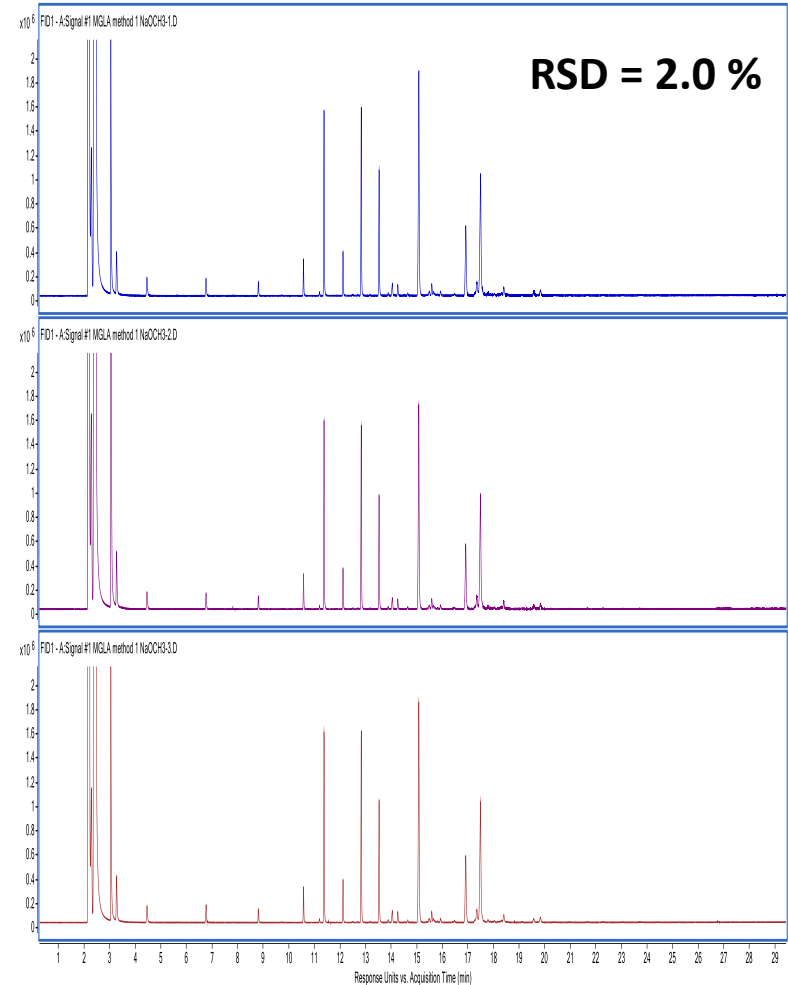
And more: automated analysis of sugars in Chocolate

Gerstel Sequence Schedule - Instrument #2

Add 1 mL H$_2$O/pyridine

centrifuge

agitation & vortex cycles

+ 500 µl oximation

+ 300 µl silylation

agitation

vortex

Add, Add, Transport, Mix, Transport, Wait, Add, Wait

25 min wait/reaction time

5 min cooldown

centrifuge

+ 450 µl isooctane

+ 450 µl water

- 200 µl extract

Add

+ 1500 µl isooctane

Sample:
100 mg of chocolate

0:00 4:00 8:00 12:00 16:00 20:00 24:00 28:00 32:00 36:00 40:00 44:00 48:00 52:00 56:00 1:00:00 1:04:00 1:08:00 1:12:00 1:16:00 1:20:00 1:24:00 1:28:00 1:32:00 1:36:00 1:40:00 1:44:00 1:48:00

No Injections

Estimated duration: 1:50:50

Fractionation & GC/LC-MS Analysis of Intact Lipids

```
┌─────────────────┐
│     Sample      │
└─────────────────┘
         │
┌─────────────────┐
│      Lipid      │
│   Extraction    │
└─────────────────┘
         │
┌─────────────────┐
│  Fractionation  │
│      (SPE)      │
└─────────────────┘
         │
    ┌────┴──────────────┐
    │  ┌─────────────────┐
    │  │    LC-QTOF      │
    │  │    (intact)     │
    │  └─────────────────┘
    │
    │  ┌─────────────────┐
    └──│     GC-MS       │
       │    (FAMES       │
       └─────────────────┘
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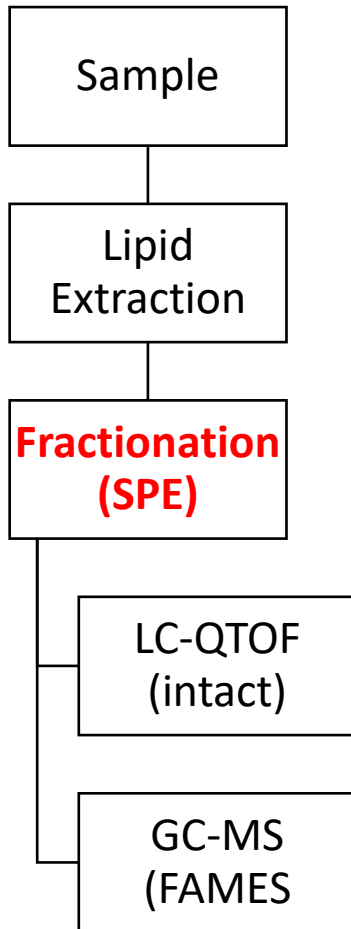

Configuration includes: SPE & mVAP (vacuum concentration)

LC-Q-TOF MS analysis of intact lipid fractions

- Bound 3-MCPD and glycidol in edible fats and oils

  - Glycidyl ester (GE) -> monoester
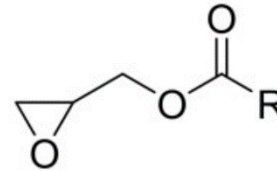
  - 2- and 3- MCPD fatty acids esters -> mono- or di-esters

*monoesters*   *diesters*
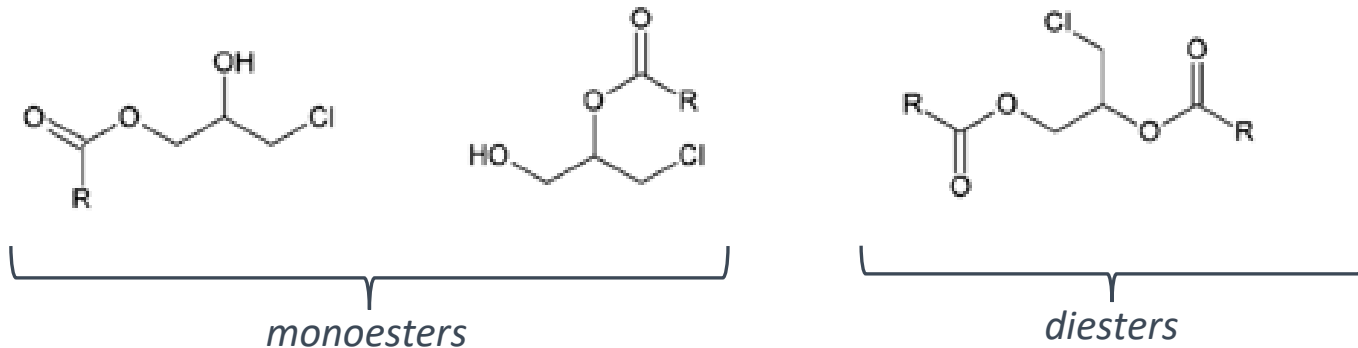
Automated sample preparation and analysis of 2-MCPD, 3-MCPD and glycidol in edible oils and fats

- MPS Robotic Dual head + GC – triple quadrupole MS

- Method:
  - Add ISTD
  - Alkaline transesterification with sodium methoxide in methanol at 10°C **(exactly 12 min)**
  - Reaction with NaBr
  - Derivatisation with phenylboronic acid
  - Removal of excess PBA with **mVAP**

- **GC-MS/MS with back-flush**
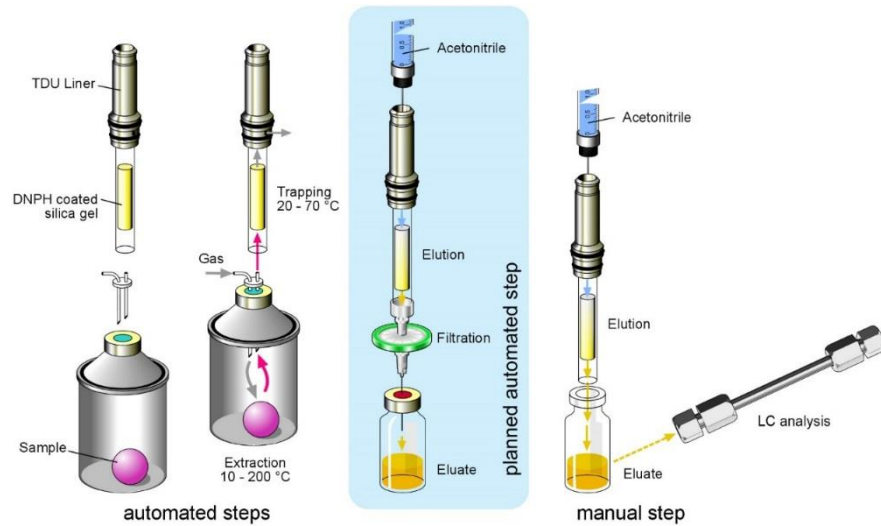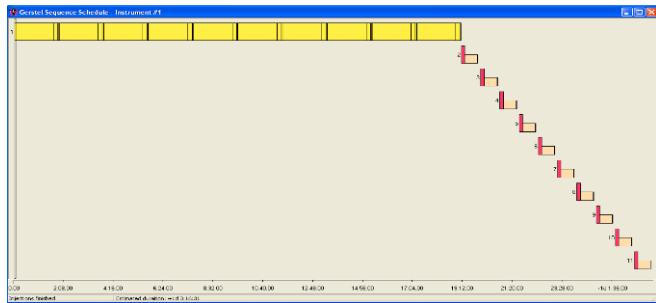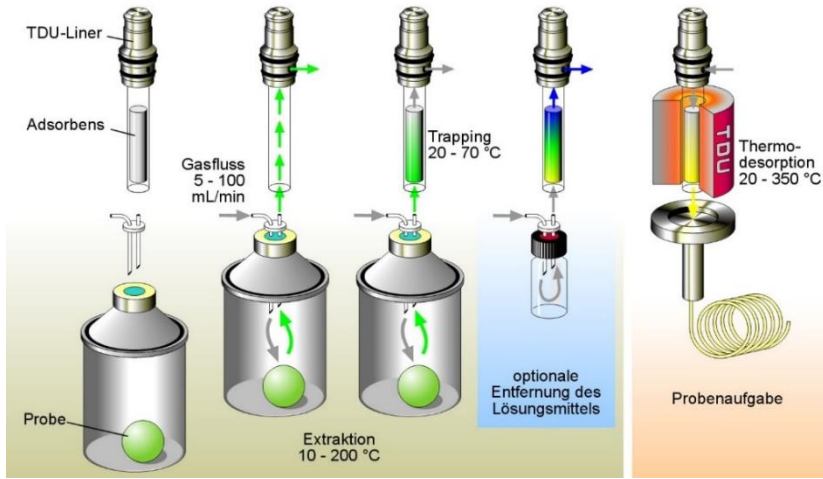
Conclusions

- Take advantage of state-of-the-art GC-MS & LC-MS
  - MS/MS, TOF: order of magnitude more sensitive
- Adapt sample preparation: Miniaturized – Automated – Green (solventless) – increased Productivity – Safer
  - Also "simple" operations can benefit from automation (preparation of mixtures, calibration solutions,…)
- Solutions available for
  - Environmental analysis
  - Food analysis
  - Analysis of natural products, essential oils, perfumes and consumer products

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