Use of automated sample preparation techniques for challenging sample by GC-MS

Sean O'Connor

- 7 Years Flavour Industry
  - GC-FID, GCMS
- 11 ½ Years Unilever SEAC
  - LC, GC, GCMS, LCMS, GCQQQ, LCQQQ MPS
- 1 Year Anatune
  - GC, GCMS, GCQQQ, GC/QTOF, MPS

Anatune

- Specialise in GC, MS and automation in many industries
- Agilent VAR
- UK Supplier of Gerstel MPS Autosampler
- Based in Girton, Cambridge

Applications Laboratory

- GC/MS
- GC/MS/MS
- GC/QTOF
- All have Dual head MPS2 Autosamplers
- Growing Team – 2 to 7 people in 18 months

Todays Talk

- Why Automate ?
- Metabolomics Derivatisation and Extraction
- Multivolatile method (MVM)

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WHY AUTOMATE ?

Why Automate ?

- We’re too busy
- Automation means losing jobs
- I have done it this way for years and it works

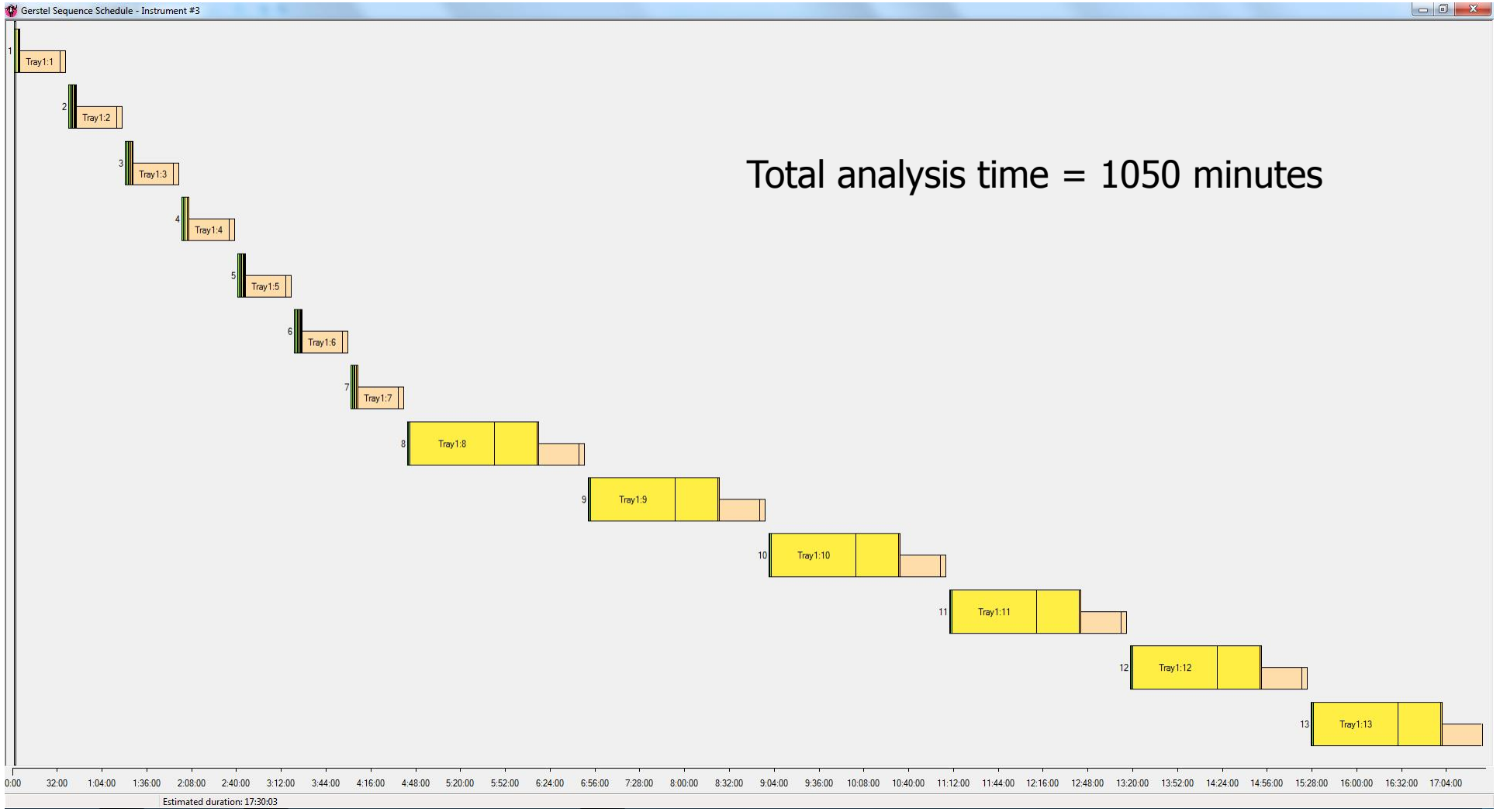
Manual method

- Prepare IS solution (5 minutes)
- Prepare calibration stock solution (5 minutes)
- Prepare 5 standards + 2 AQC (30 minutes)
- Add 100 mL of sample to each extraction flask (1 minute per sample)
- Add 200 µL of IS solution to each sample (10 seconds per sample)
- Add 20 mL of extraction solvent (1 minute per sample)
- Shake for 1 hour and allow to separate 30 minutes
- Remove extract from extraction vessel and transfer to vial for analysis (30 seconds per sample)
- Injection and GC run (30 minutes)
- Dispose of waste and clean glassware for next analysis (30 minutes)

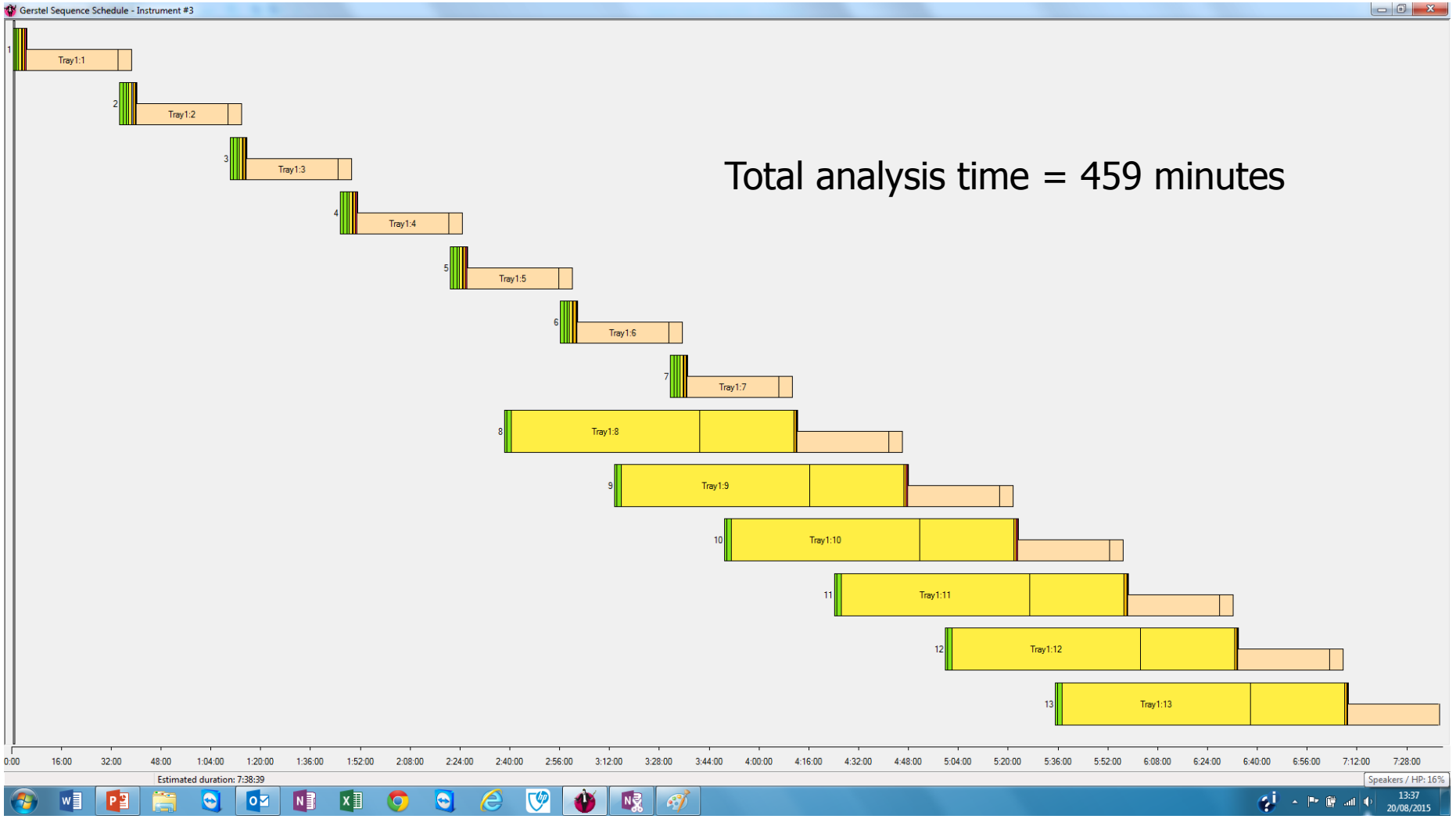
Why Automate ?

- Prepare IS solution (5 minutes)
- Prepare calibration stock solution (5 minutes)
- Add 5 mL of sample to each 10 mL vial (1 minute per sample)
- Prepare 5 standards + 2 AQC (30 minutes)
- Add 10 µL of IS solution to each sample (10 seconds)
- Add 1 mL of extraction solvent (20 seconds)
- Shake for 1 hour and allow to separate 30 minutes
- Directly inject from extract layer (30 minute run time)
- Dispose of vials (30 seconds)

No Prep Ahead

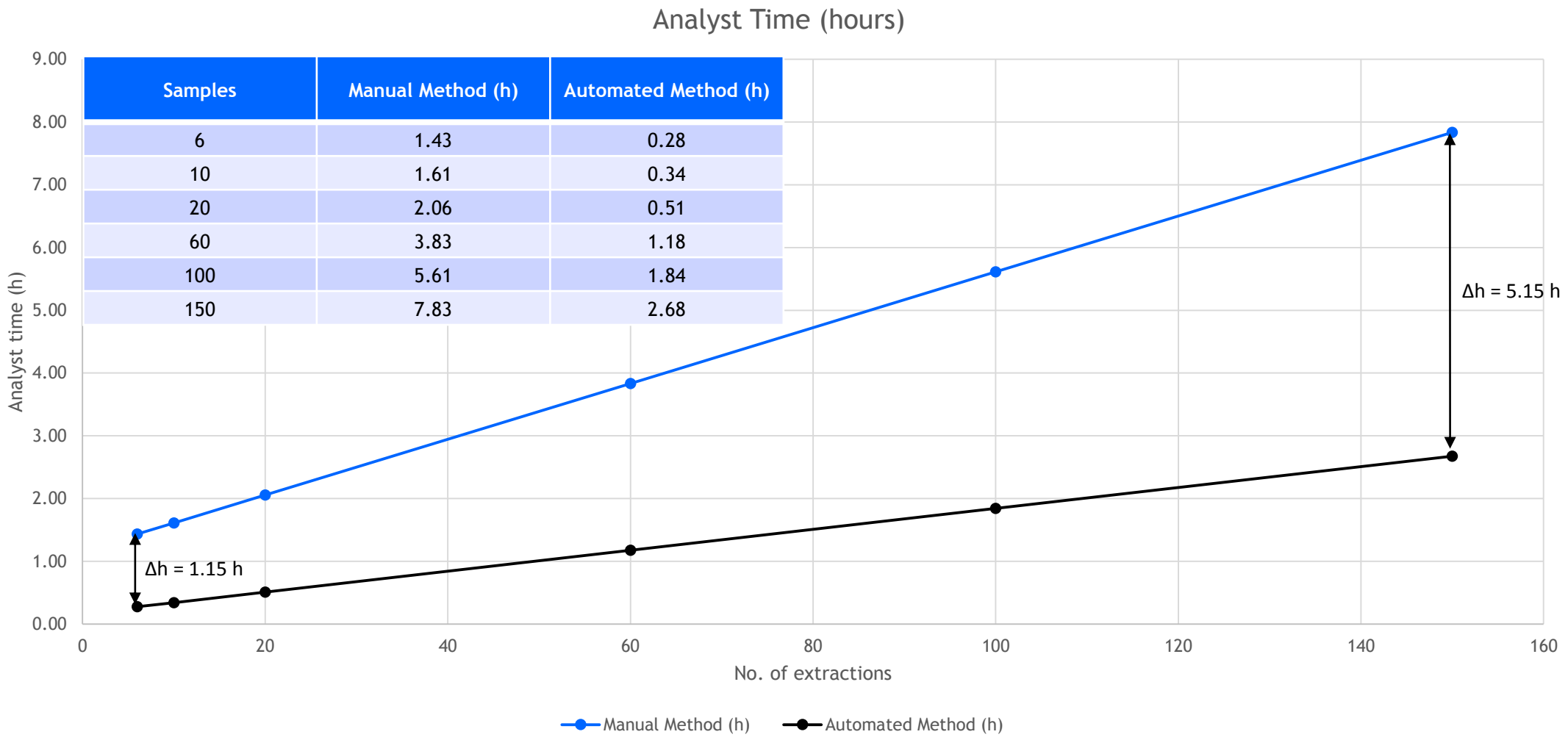


With Prep Ahead



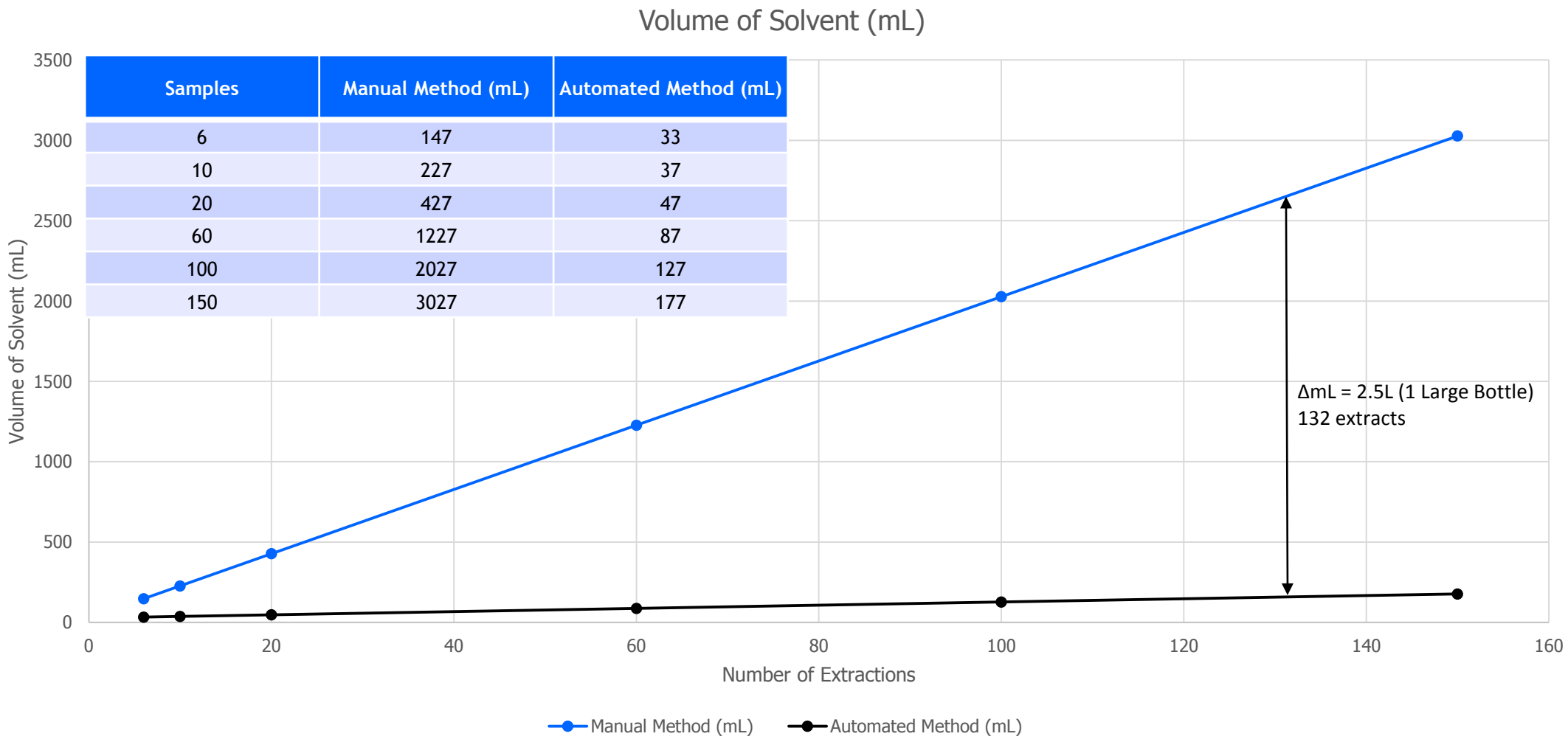
Comparison of Analyst's Time

Samples	Manual Method (h)	Automated Method (h)
6	1.43	0.28
10	1.61	0.34
20	2.06	0.51
60	3.83	1.18
100	5.61	1.84
150	7.83	2.68



Solvent Saving

Samples	Manual Method (mL)	Automated Method (mL)
6	147	33
10	227	37
20	427	47
60	1227	87
100	2027	127
150	3027	177



Why automate ?

Manual Preparation

- Preparation restricted to working hours
- Different people have different ideas as to how things are done
- Samples and standards are prepared all at the same time
- Glassware clean up required before next use
- Exposure to solvents a potential hazard / safety risk

Automated Preparation

- Works 24/7
- Consistency
- Samples are prepared just in time for analysis
- Solvent (Cost) saving – Every 132 extracts (on method shown) saving a 2.5 L bottle of solvent (£50-£100)
- Analysis done all in vial – fewer losses
- Exposure to solvents reduced

What tools do we have ?

MultiPurpose Sampler MPS

Cooled Injection System CIS

Automated Liner EXchange ALEX

easy Liner Exchange eLEX

MultiFiber EXchange MFX

Thermal Desorption System TDS

Thermal Desorption Unit TDU

Automated TDU Liner Exchange ATEX

Twister

Dynamic Headspace DHS

TDU PYRO

µFlowManager

Selectable 1D/2D GC/MS

Olfactory Detection Port OPD

Preparative Fraction Collector PFC

Disposable Pipette Extraction DPX

MultiPosition Evaporation Station mVAP

MAESTRO Prep Ahead

Solid Phase Extraction SPE

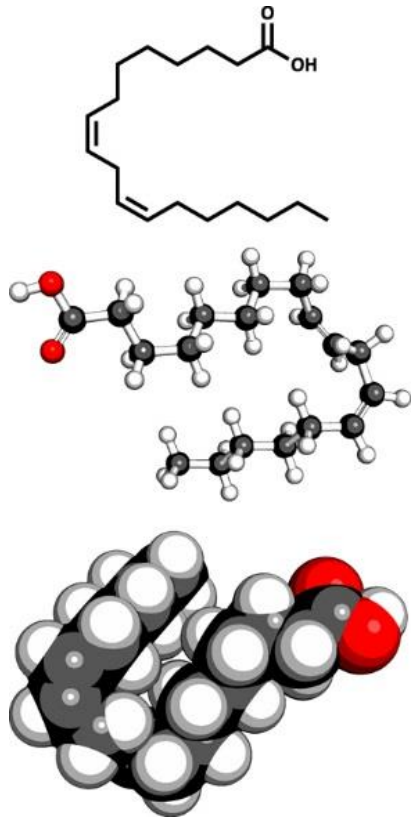
Instrument Top Sample Preparation ITSP

Filtration

Balance

mVorx

Maestro Software



OMIC DERIVATISATION AND EXTRACTION

'Omics Requirements

- Reliable
- Reproducible
- Lots of data points

Omics Extraction and Derivatisation

Omics Extraction and Derivatisation

Omics Extraction and Derivatisation

Weigh (Manual)

Accurately weigh aproximately 5-6 mg of sample

Methylation (Automated)

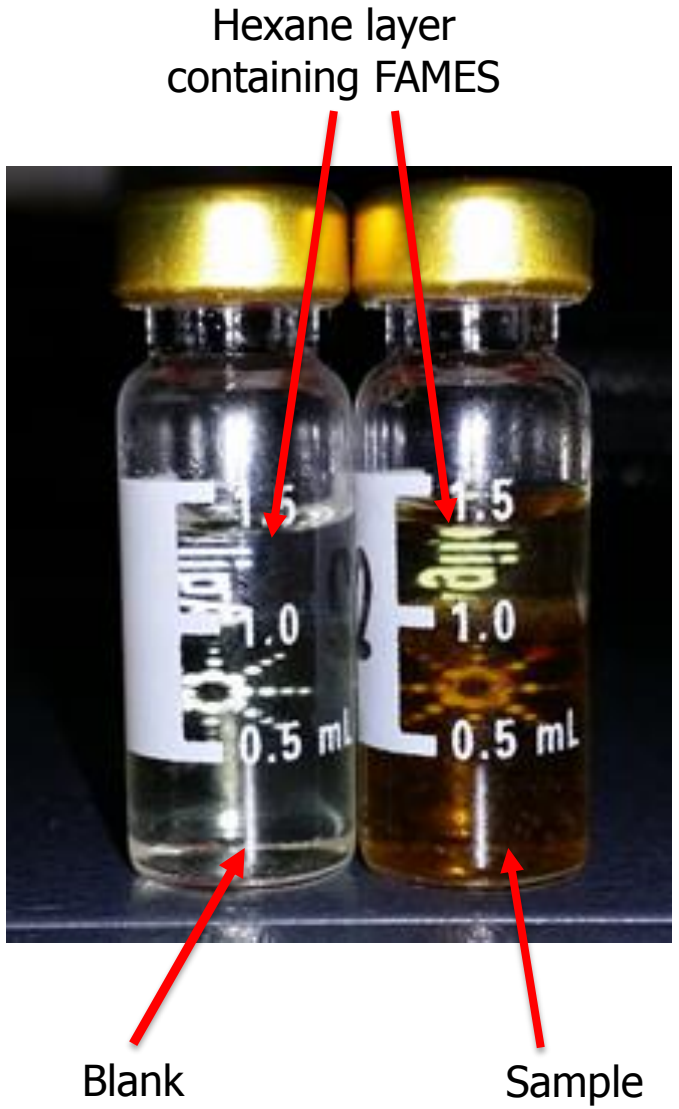
Add 500 µL of methanolic hydrochloric acid. Agitate at 500 rpm and 70 °C for 15 minutes. Allow to cool.

Liquid – liquid extraction (Automated)

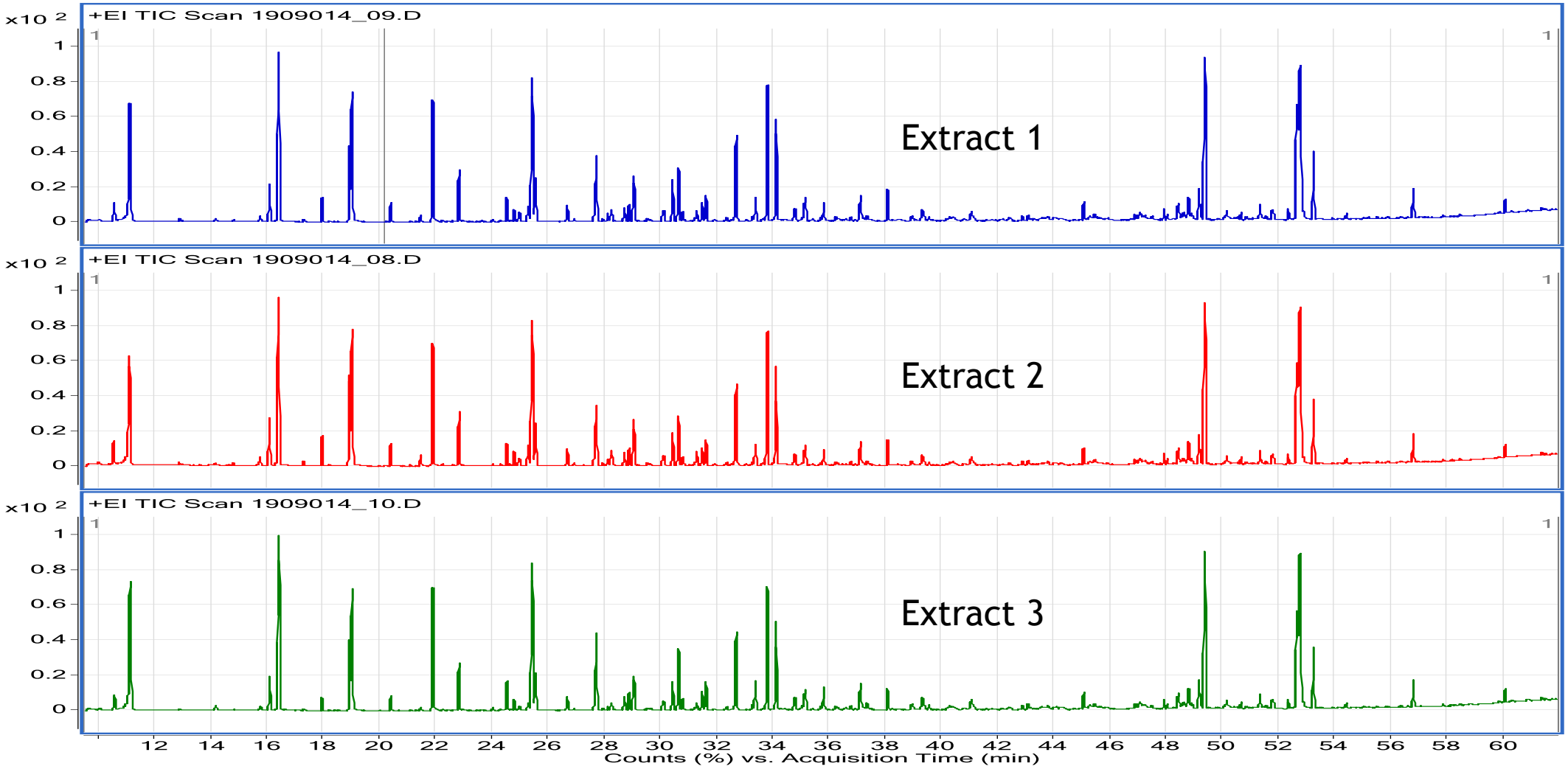
Add 500 µL of hexane and 500 µL of water.

Large Volume Injection (Automated)

A 10 µL injection was made utilising the CIS injector from the top layer

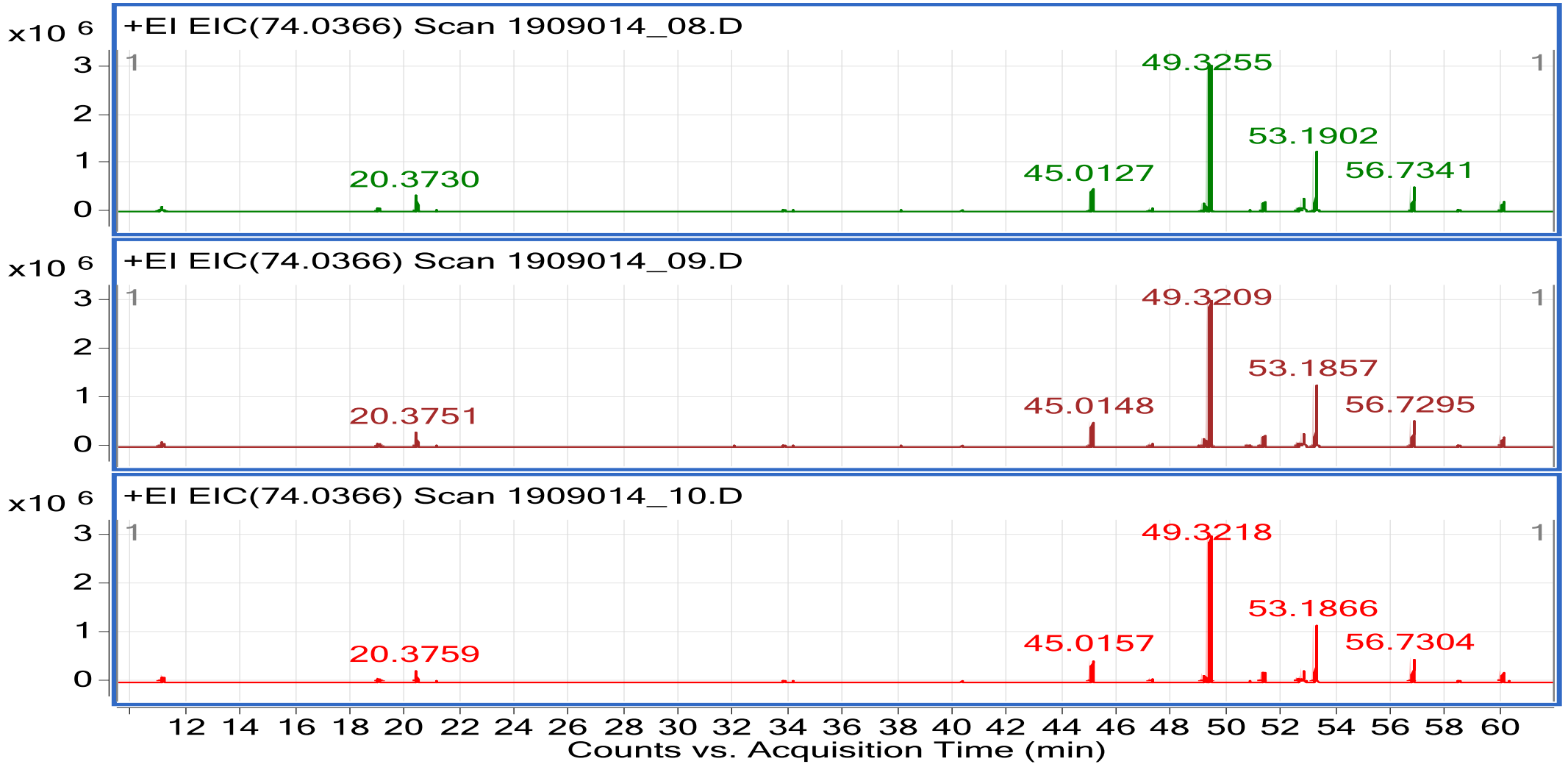


Omics Extraction and Derivatisation



Total ion chromatogram of the hexane layer from the three extracts.

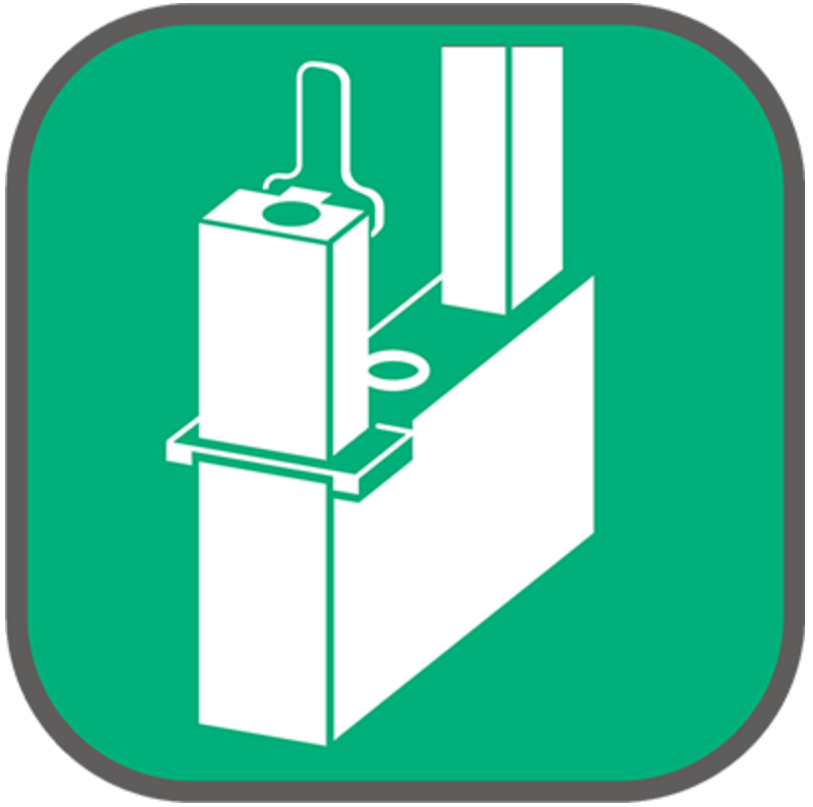
Omics Extraction and Derivatisation



Extracted Ion Chromatogram of m/z = 74.0377 (McClafferty re-arrangement of the ester grouping)

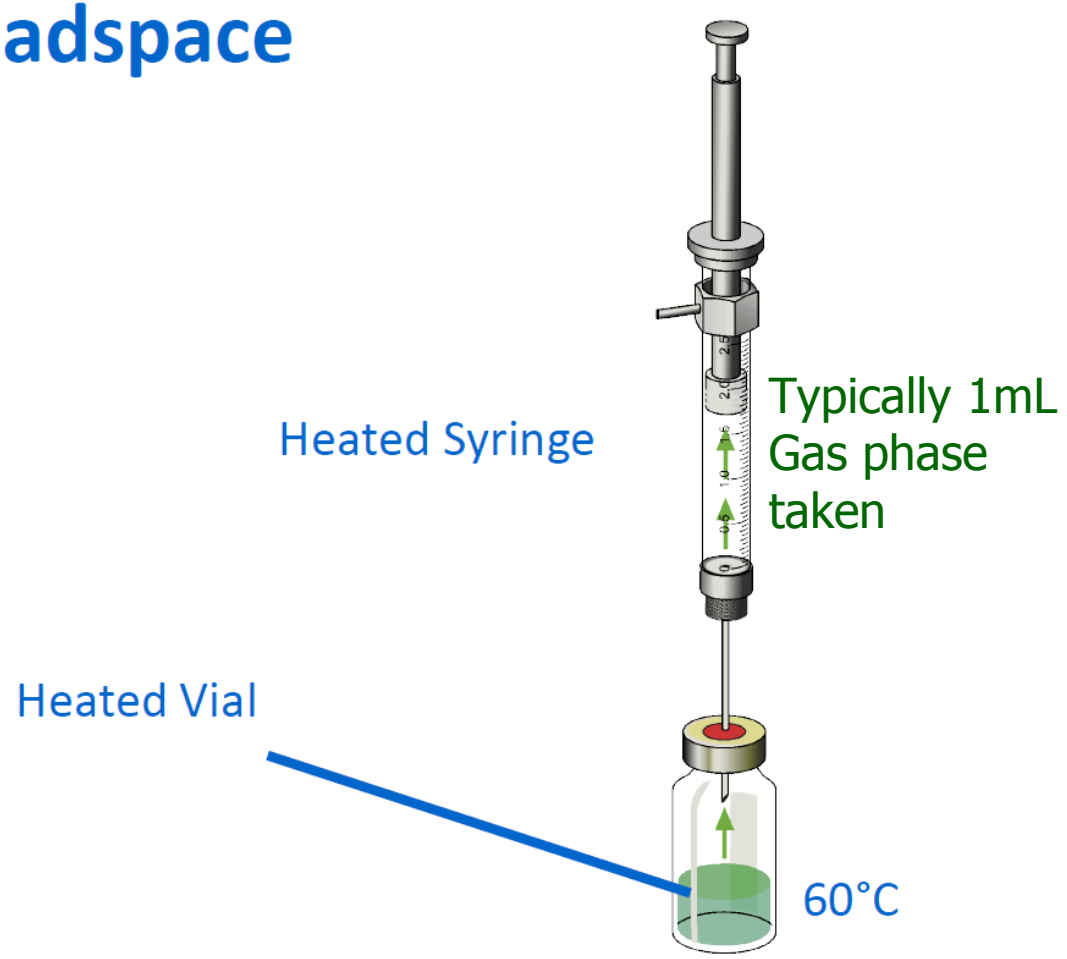
Omics Extraction and Derivatisation

FAME	% CV
Methyl tetradecanoate (C14:0)	6.9
Methyl hexadecanoate (C16:0)	4.7
Methyl octadecanoate (C18:0)	6.2
Methyl eicosanoate (C20:0)	5.8
Methyl docosanoate (C22:0)	3.9

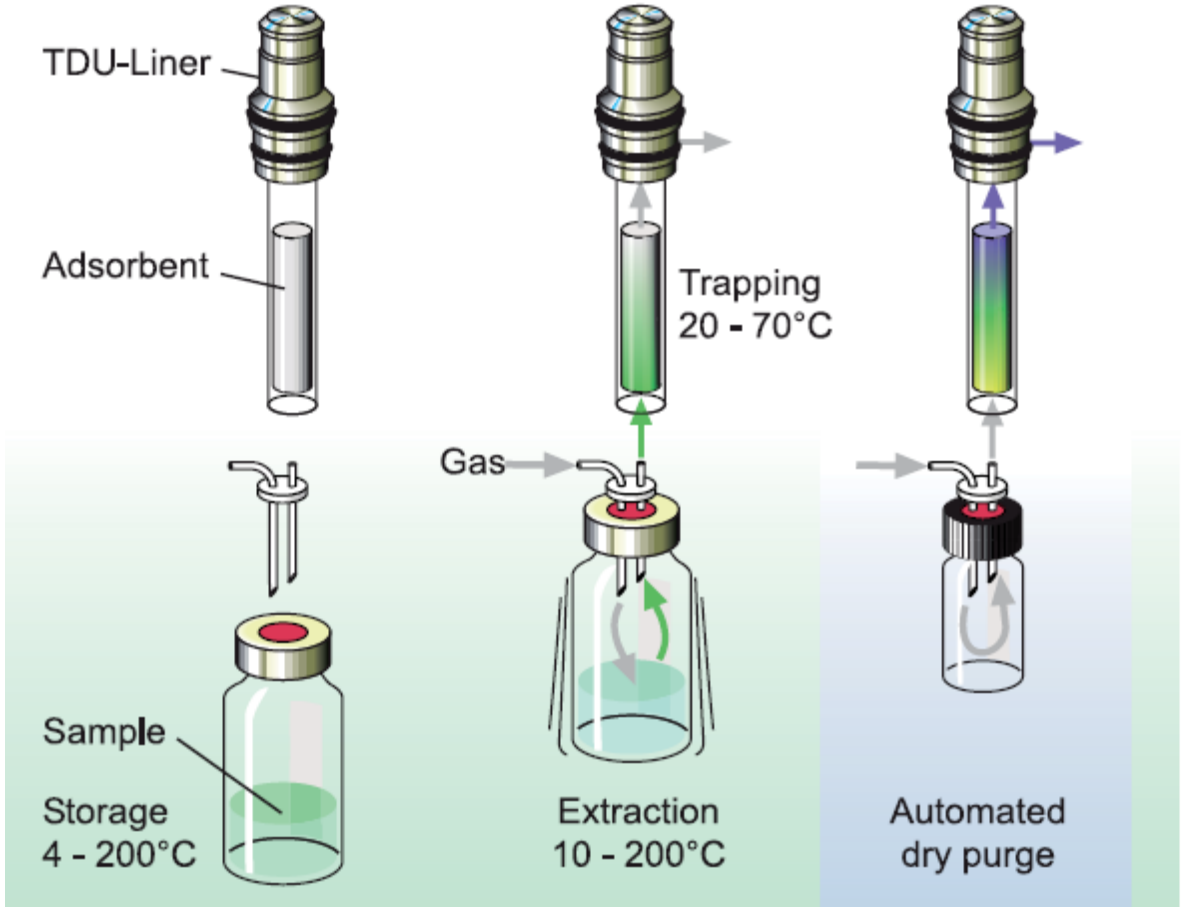


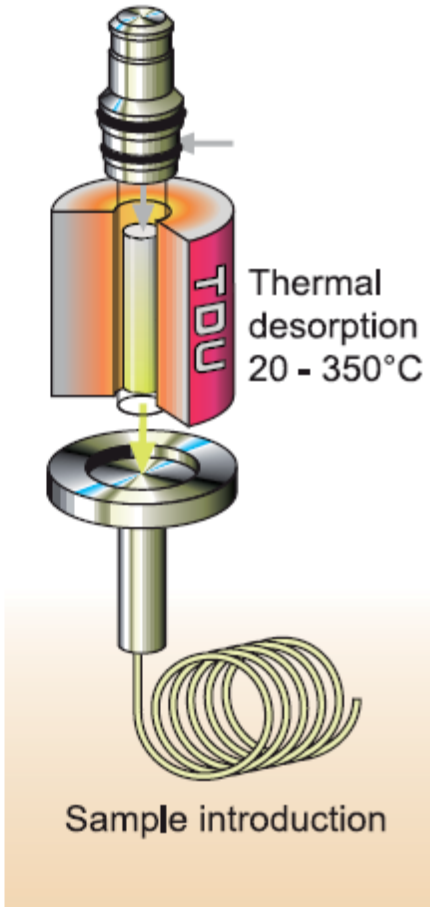
DYNAMIC HEADSPACE AND MULTI-VOLATILE METHOD

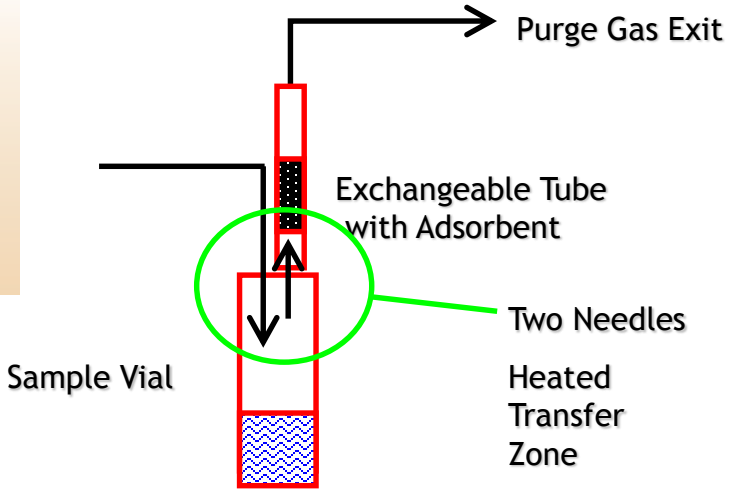
Static Headspace



Dynamic Headspace (DHS)

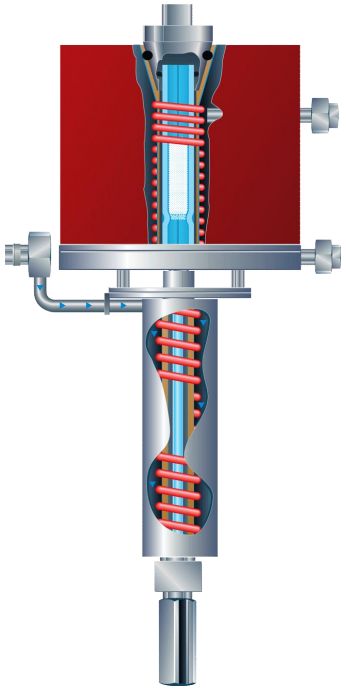




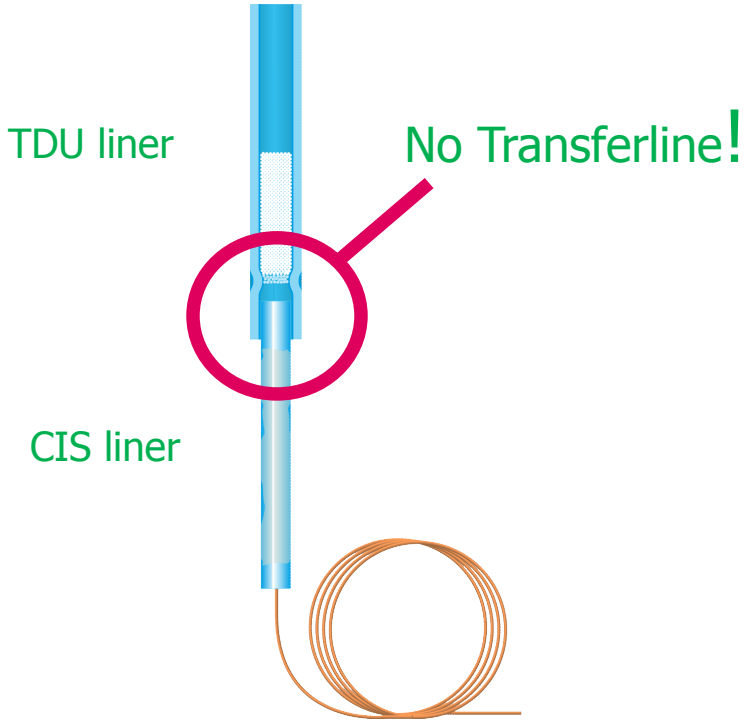


Thermal Desorption Unit (TDU) and Cooled Inlet System (CIS)

Thermal Desorption Unit



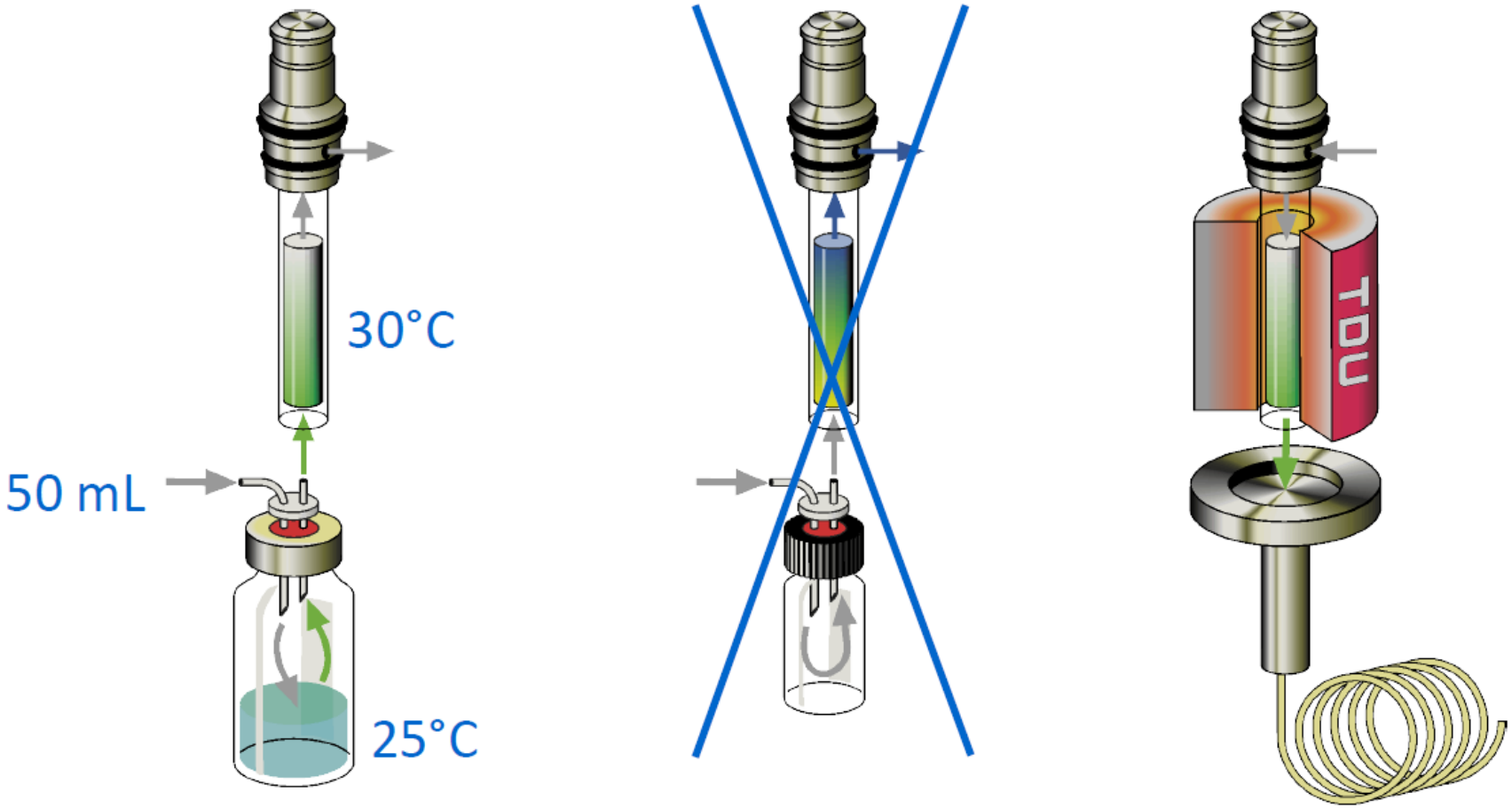
Cooled Injection System



DHS – Very Volatile Analytes

Dynamic Headspace

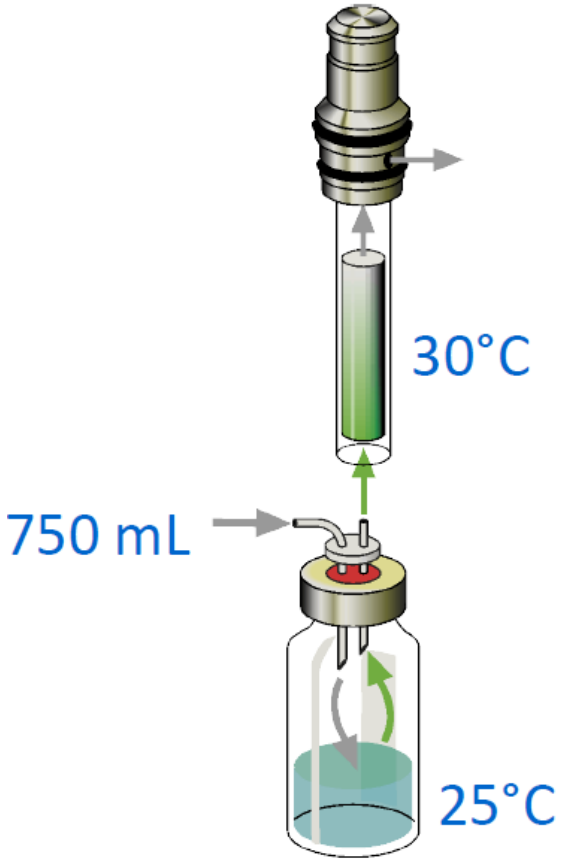
Method 1: Very Volatile Analytes

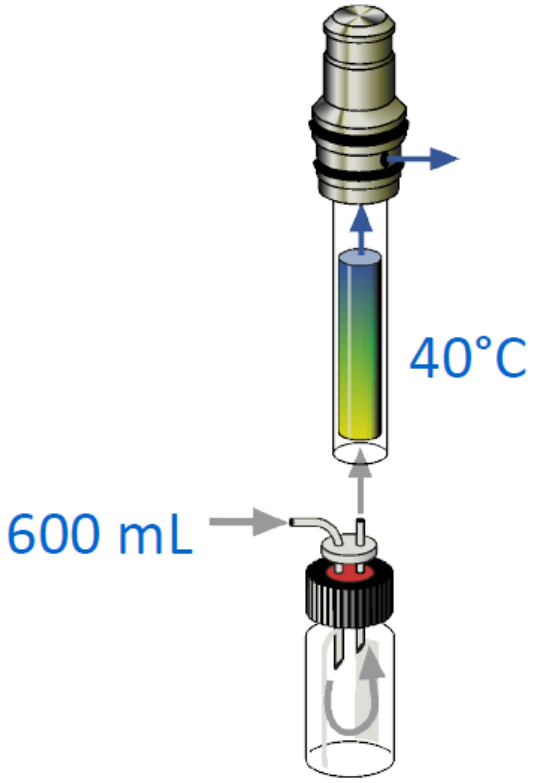


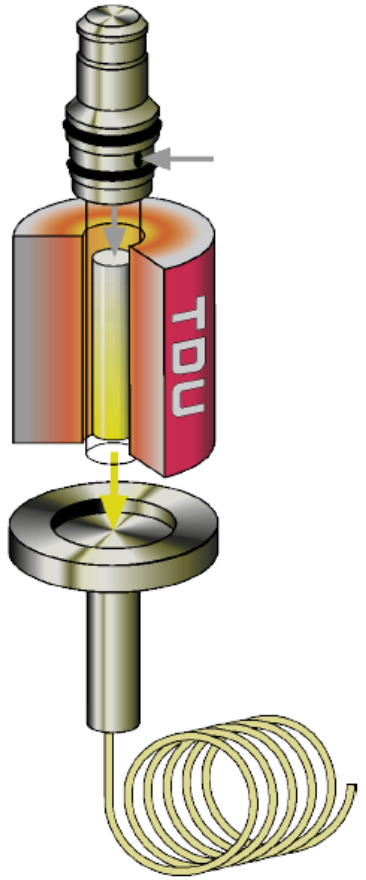
Dynamic Headspace – Volatile or Semi Volatile Analytes

Dynamic Headspace

Method 2: Volatile or Semi Volatile Analytes



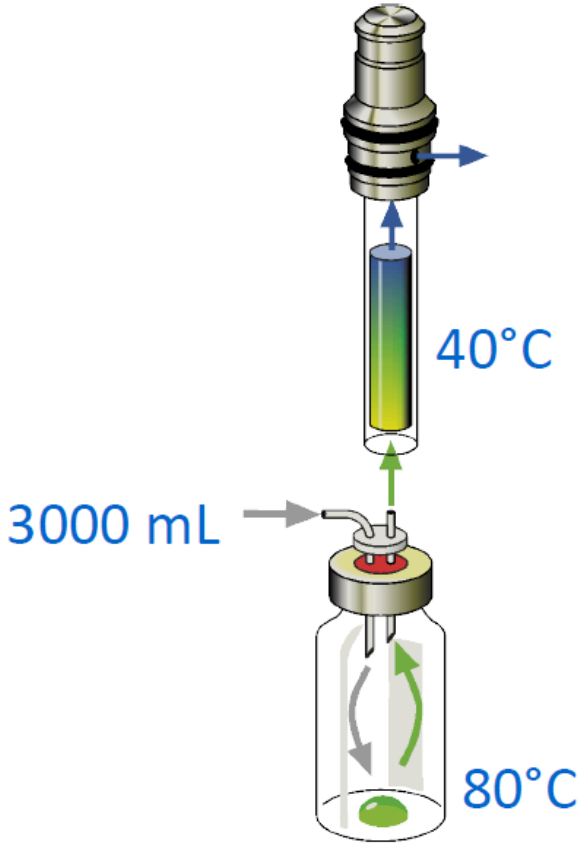


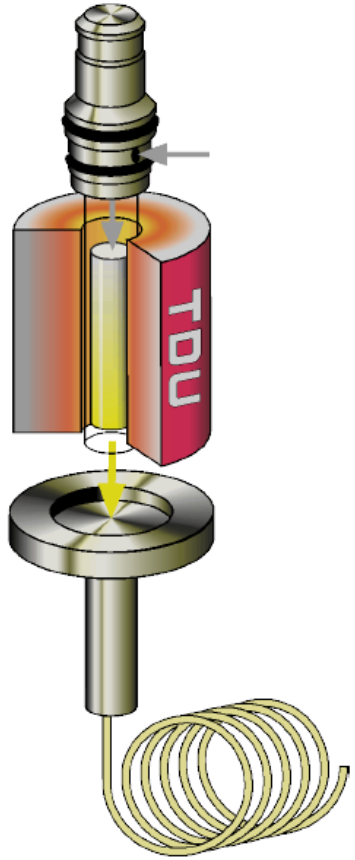


Fully Evaporative Technique - FET

Dynamic Headspace

Method 3: Volatile, non volatile and hydrophillic analytes





Multivolatile method

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Multi-volatile method for aroma analysis using sequential dynamic headspace sampling with an application to brewed coffee☆

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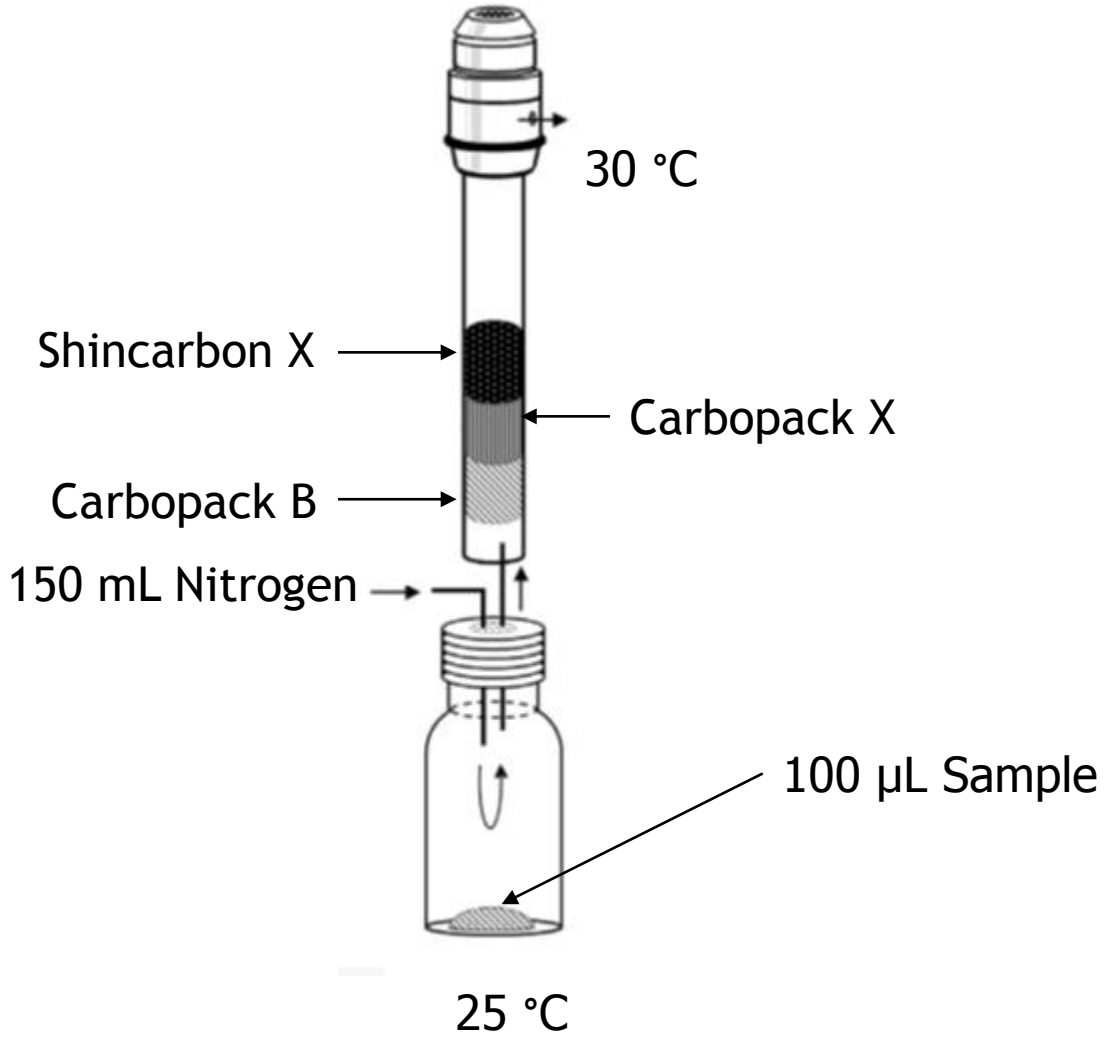
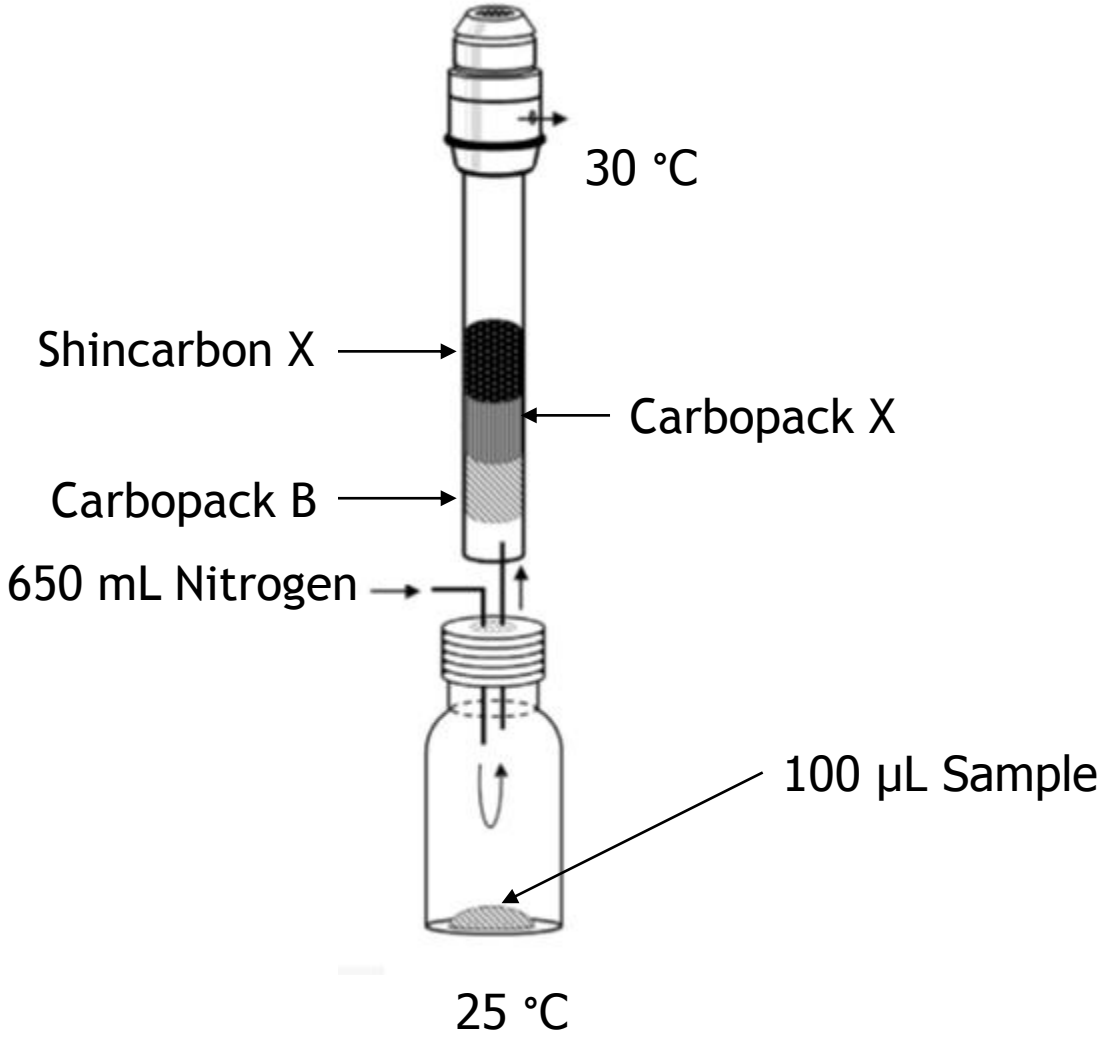
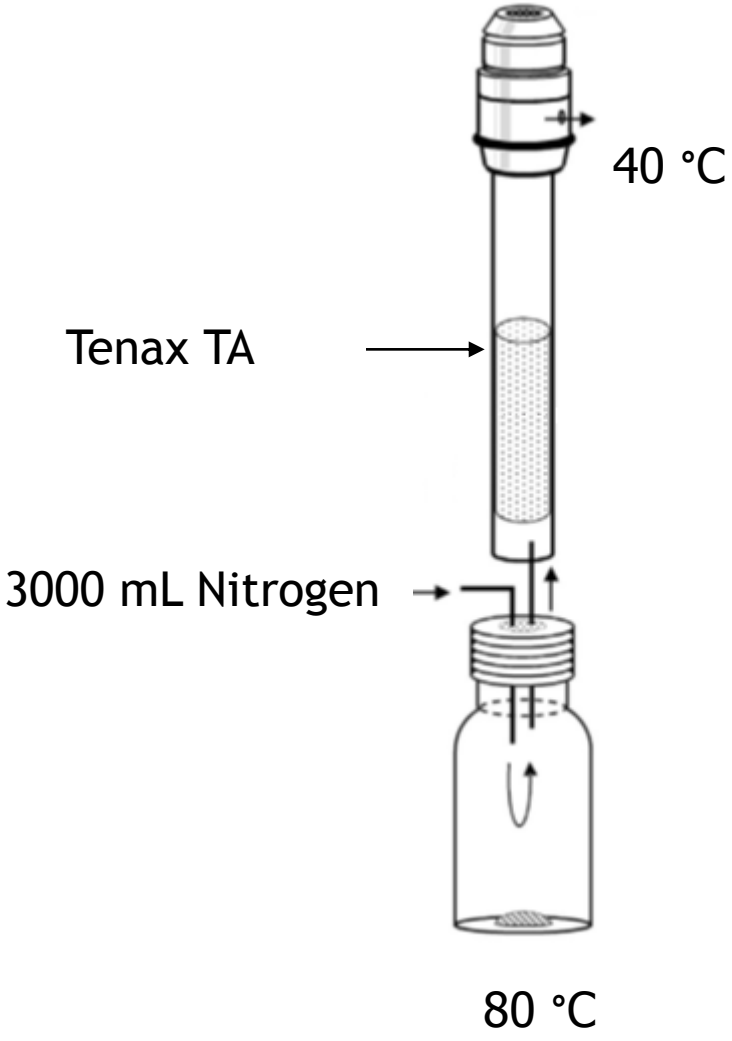
Trap 1 – Very Volatile Components



Trap 2 – Volatile/Semi Volatile Compounds



Trap 3 - FET



Multivolatile Method

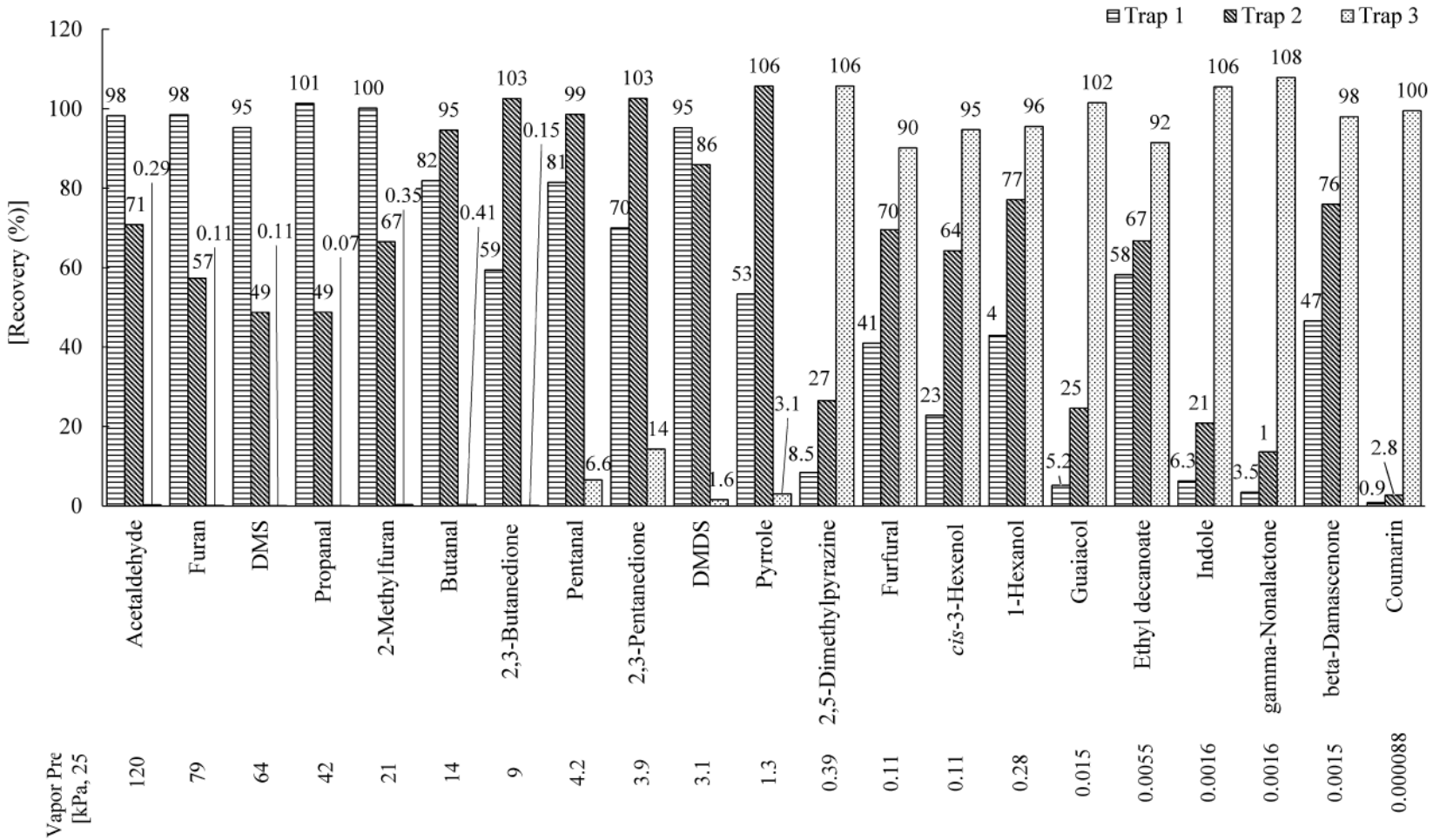
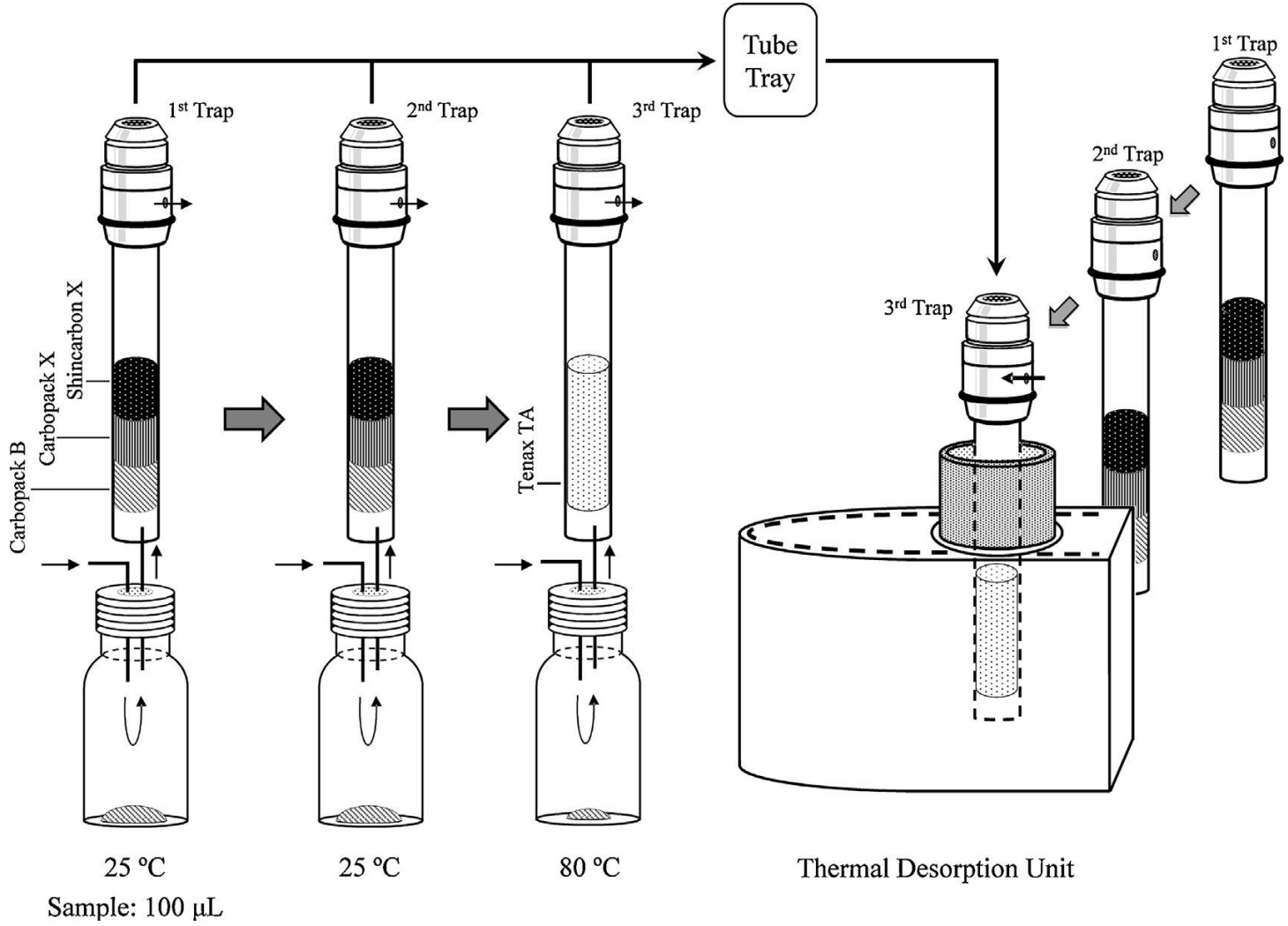


Fig. 3. Comparison of recoveries between three DHS sampling conditions for the test aroma compounds in 100 μL of water spiked at 100 ng mL^{-1}.

Multivolatile Method



Multivolatile Method

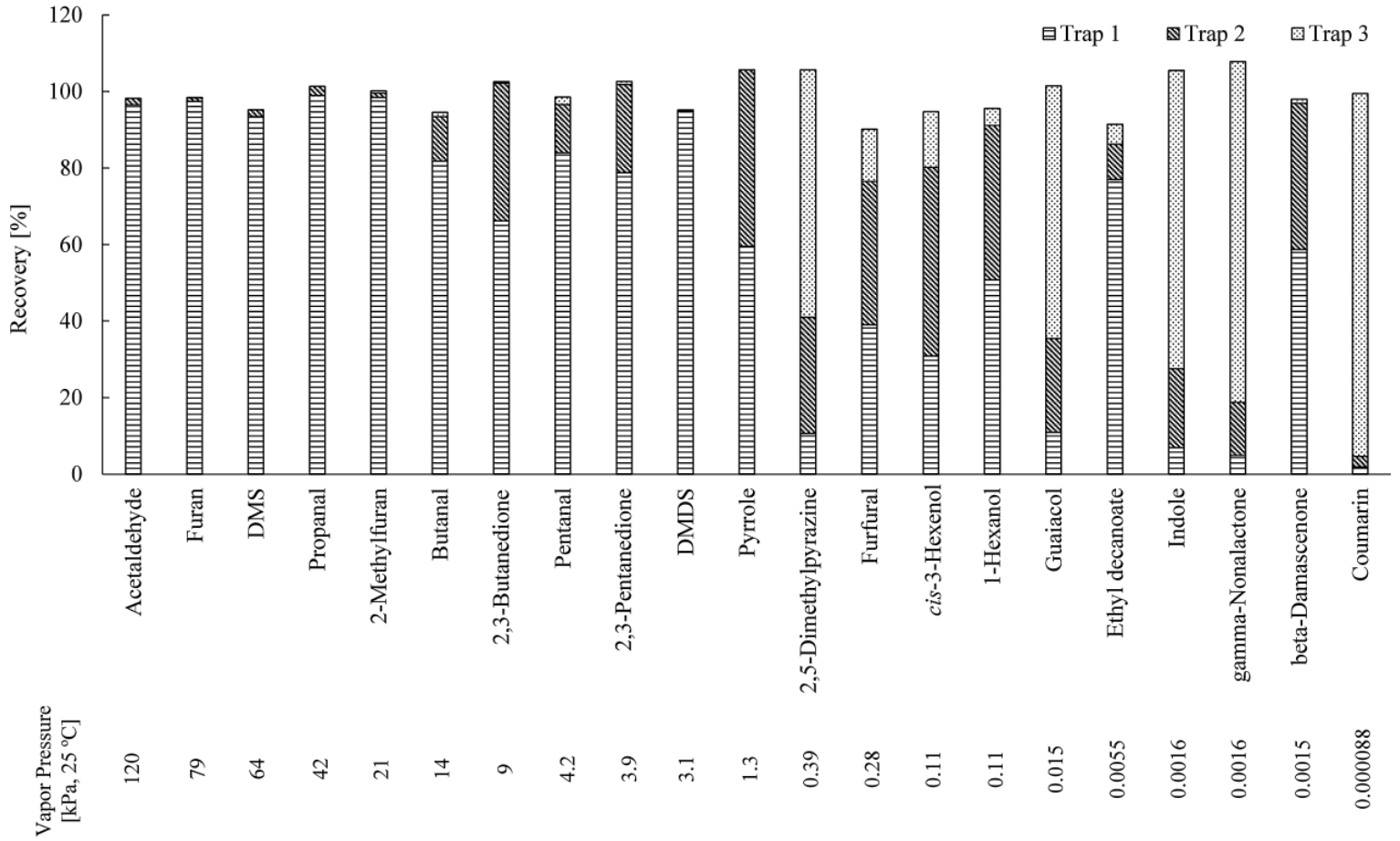


Fig. 4. Total recoveries (sum of the recoveries obtained from the three DHS conditions and three individual thermal desorption-GC-MS analysis) for the test aroma compounds in 100 μL of water spiked at 100 ng mL^{-1}.

Conclusions – Part 1

- Automation can save technician time, solvent and therefore money
- Automation can increase precision and accuracy
- Automation can reduce contact with hazardous chemicals and therefore is safer

Conclusions Part 2

- Automation can produce reliable results
- Automation can provide reproducible results
- Automation working with Prep Ahead and 24/7 schedule can produce many data points

Conclusions Part 3

- DHS is can be used to detect low trace level concentrations

- MVM is excellent technique for the extraction of compounds in aqueous matrices from the very volatile (acetaldehyde) to the semi volatile (Vanillin, Coumarin)

Acknowledgements

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Thank You

Any Questions ?