Automation of Sample Preparation using a GERSTEL MPS2

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

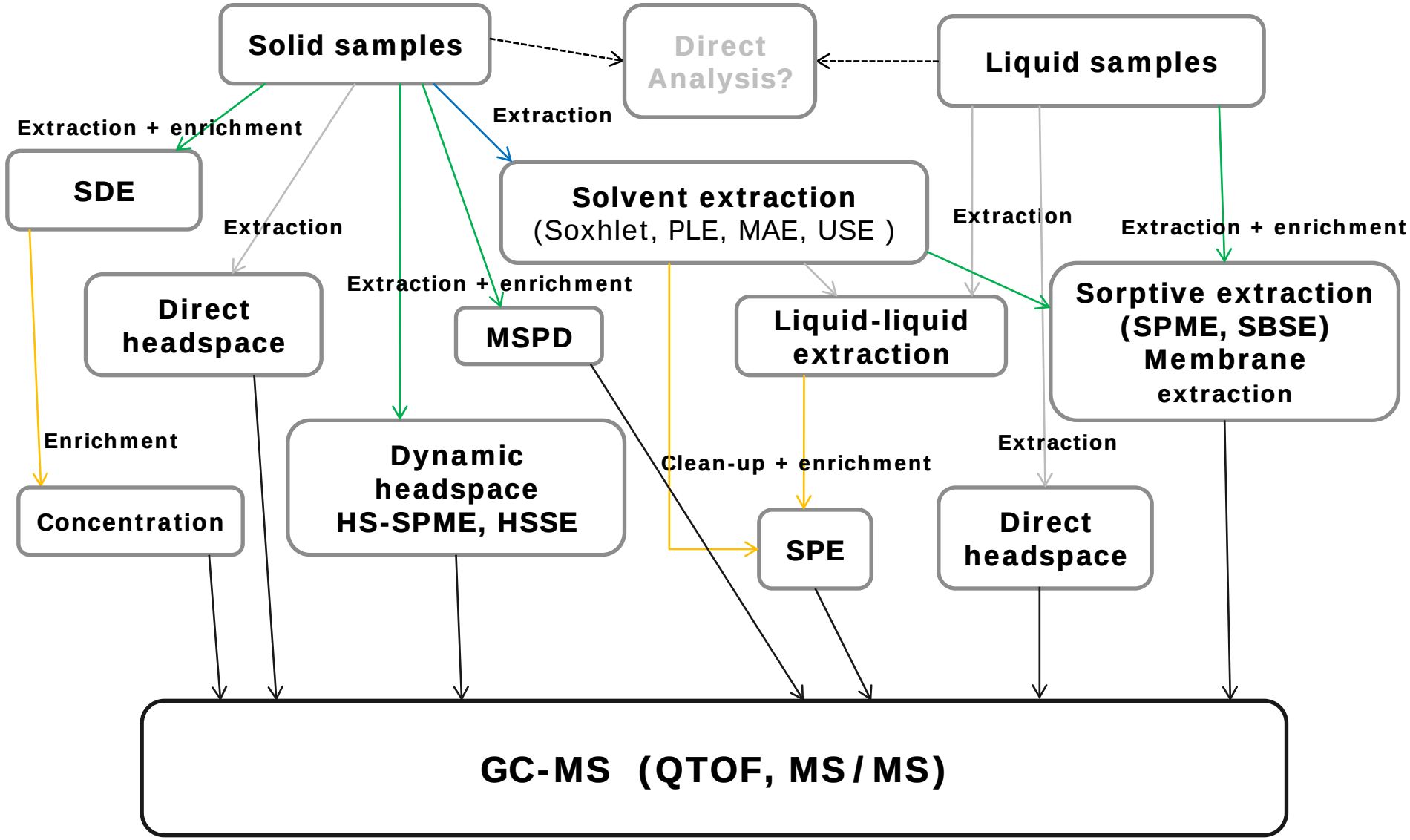
Anatune

- Specialise in GC, MS and automation in many industries
- Agilent VAR
- UK Supplier of Gerstel MPS Autosampler
- Based in Girton, Cambridge
- 20 Years old next month !!

Applications Laboratory

- GC/MS
- GC/MS/MS
- GC/QTOF
- All have Dual head MPS2 Autosamplers
- Growing Team – 2 to 6 people in 18 months

Sample preparation



Todays Talk

- Why Automate ?
- How do we automate ?
- Demo : Automated Extraction and analysis of Acid Herbicides
- Demo : Methanolic Extraction of Soil

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

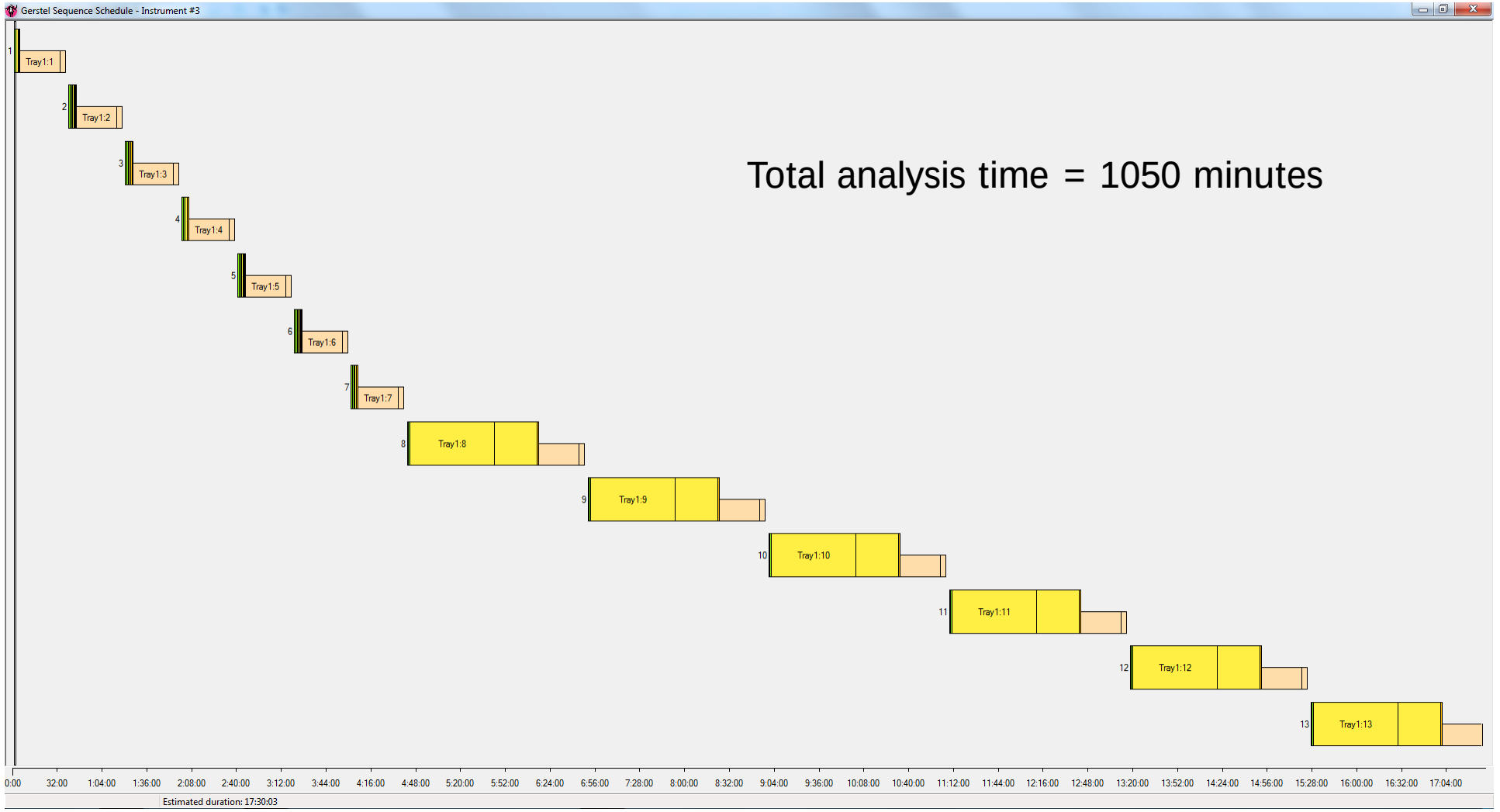
- Prepare IS solution (5 minutes)
- Prepare calibration stock solution (5 minutes)
- Prepare 5 standards + 2 AQC (30 minutes)
- Add 100 mL of sample to each extraction flask (1 minute per sample)
- Add 200 µL of IS solution to each sample (10 seconds per sample)
- Add 20 mL of extraction solvent (1 minute 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 ?

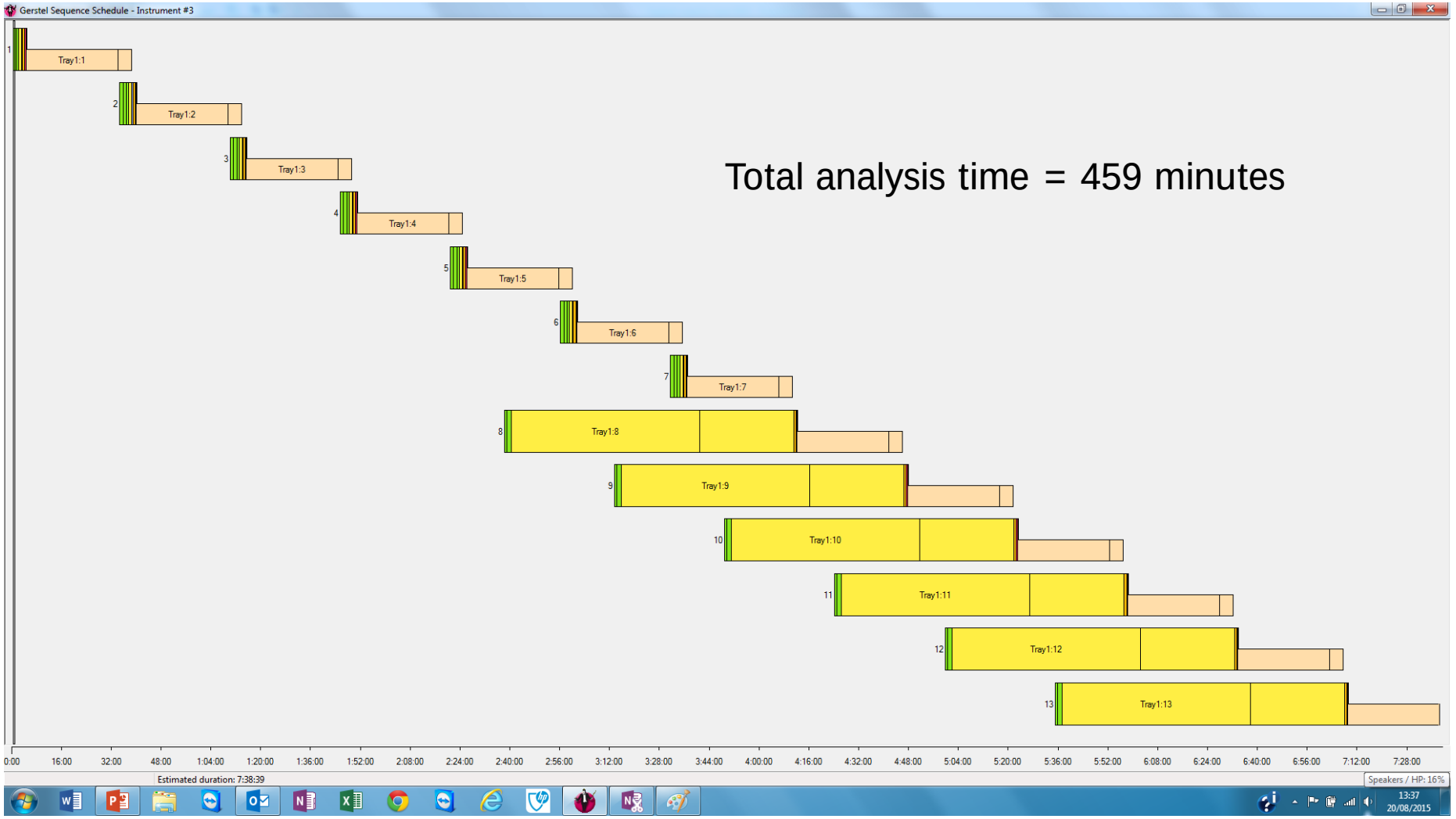
- Prepare IS solution (5 minutes)
- Prepare calibration stock solution (5 minutes)
- Add 5 mL of sample to each 10 mL vial (1 minute per sample)
- Prepare 5 standards + 2 AQC (30 minutes)
- Add 10 µL of IS solution to each sample (10 seconds)
- Add 1 mL of extraction solvent (20 seconds)
- Shake for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

Gerstel Maestro Software

No Prep Ahead

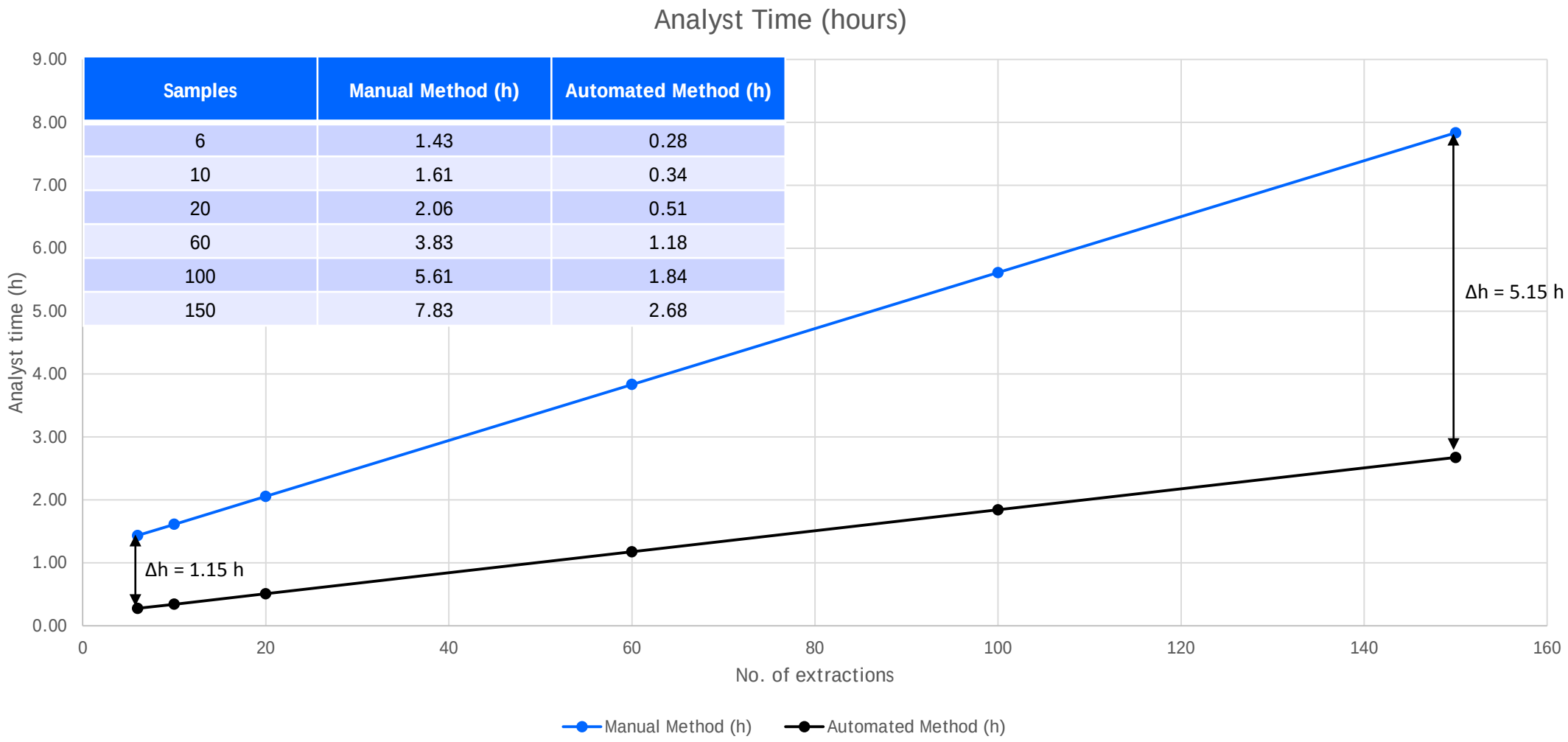


With Prep Ahead



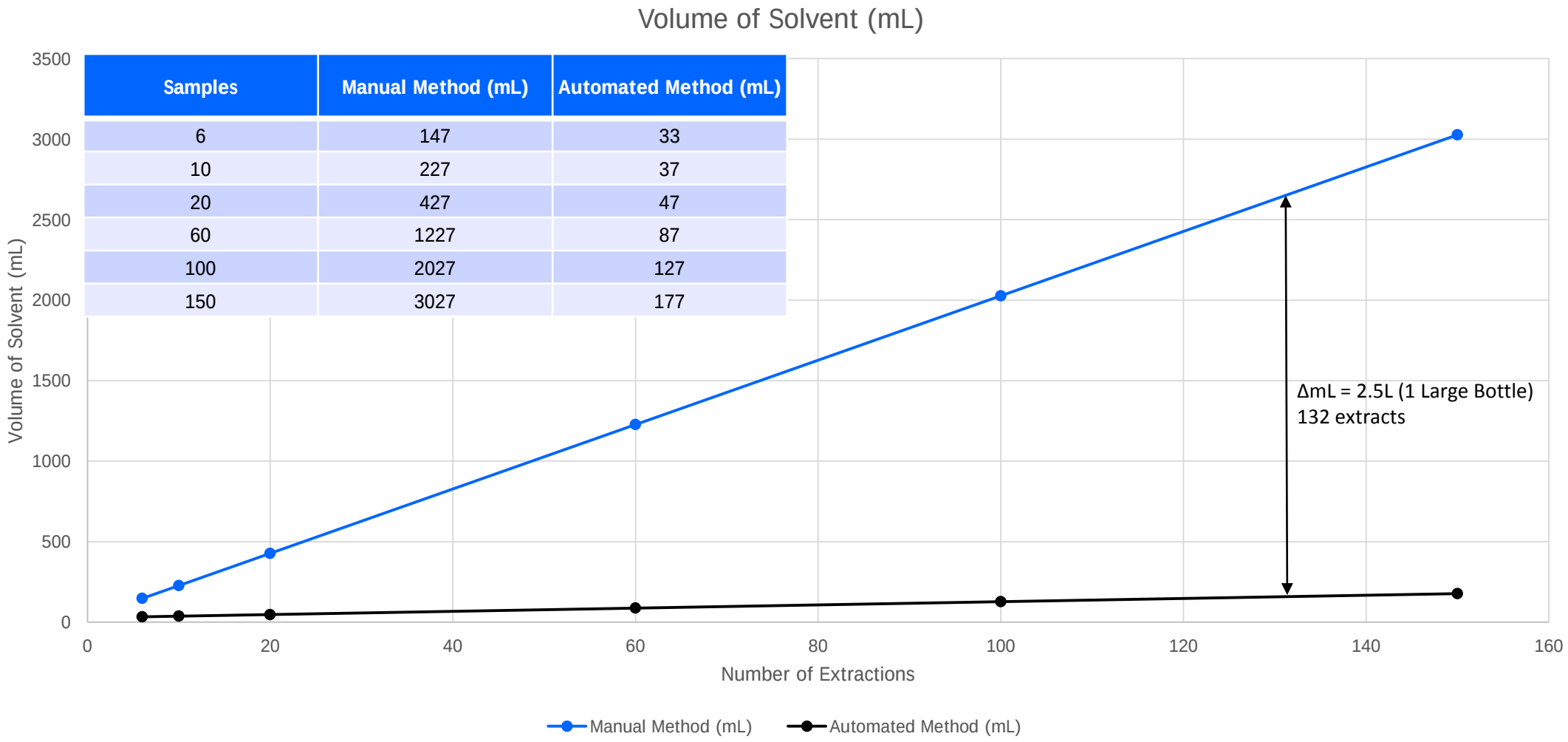
Comparison of Analyst's Time

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

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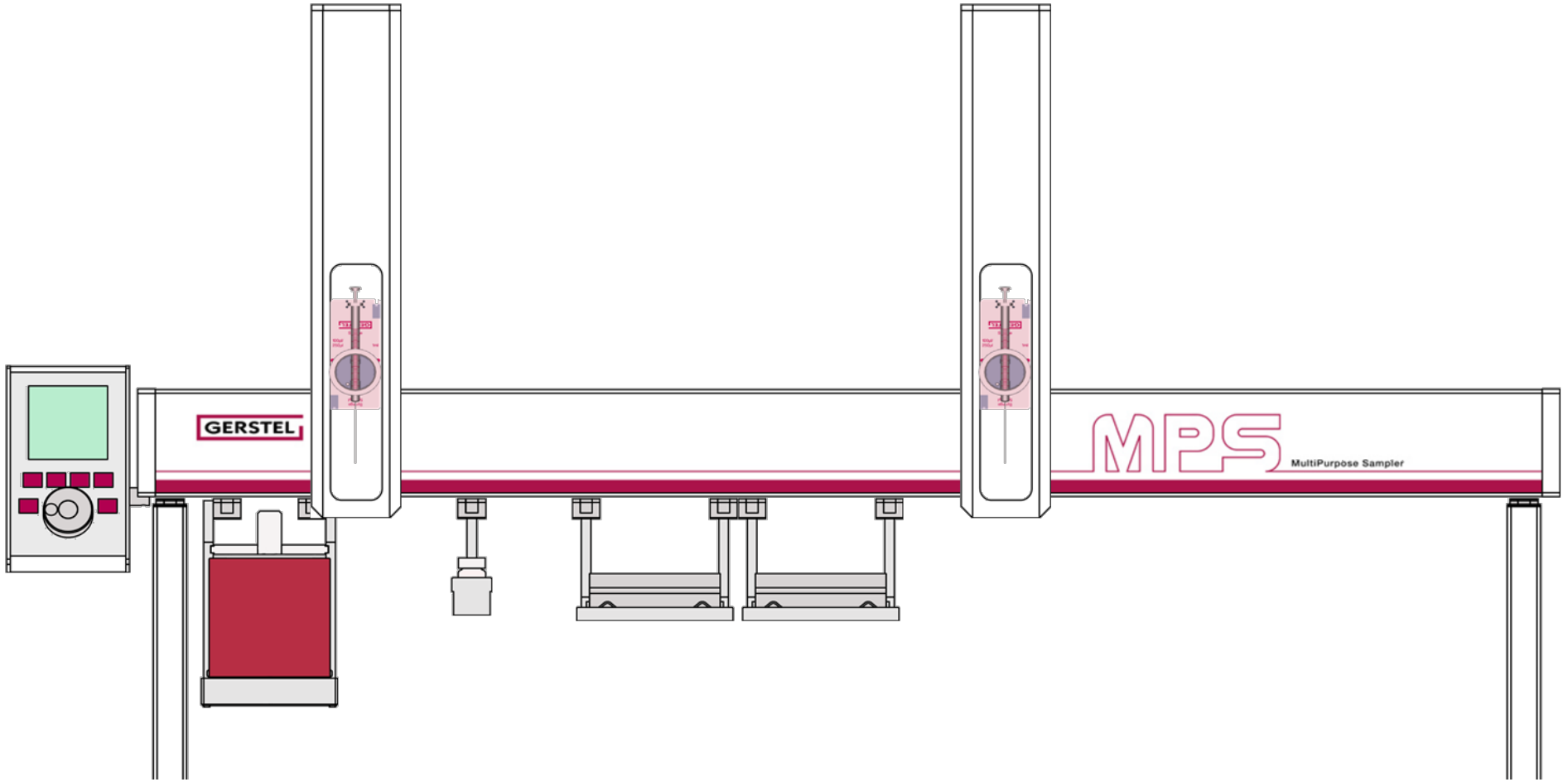
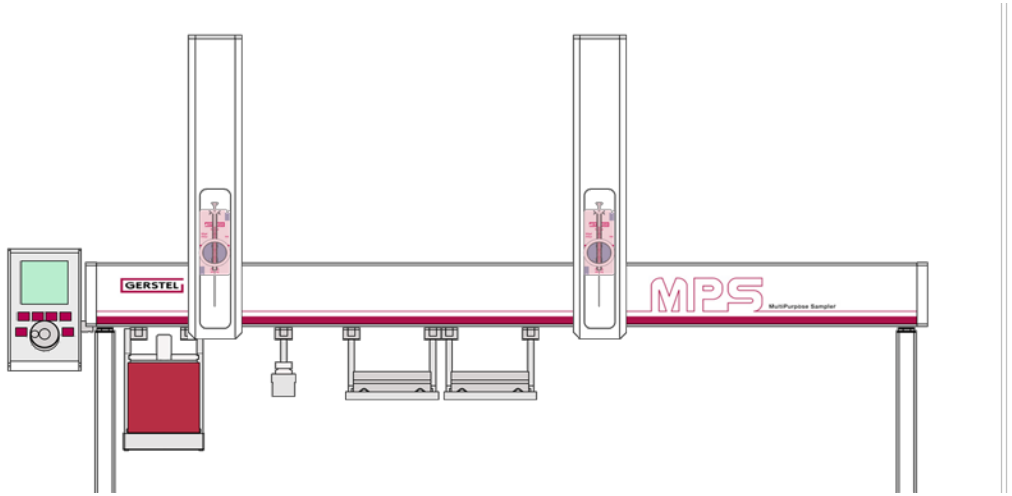
HOW TO AUTOMATE ?

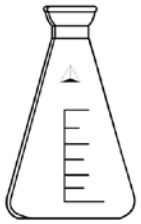
Simplest Dual Head MPS2



What can we automate with the simplest MPS2 ?

- Standard preparation
- Simple derivatisation
- Dilution
- Basic liquid extraction of easily accessible compounds





- add 20mL sample
- add 2 mL HCl 1N
- add 200 uL ISTD 1g/L
- add 2g NaCl
- add 4mL Hexane/DCM
- mix for 20 min @ 400 rpm
- recover org.Phase in GC vial

- add 5mL sample
- add 500 uL HCl 1N
- add 300 uL ISTD
- add 500 mg NaCl
- add 1mL Hexane/DCM
- mix for 20 mins @ 400 rpm
- recover org.Phase in GC vial

=> syringe: 2.5 mL

extr. vial w/ 500 mg NaCl

add 5mL sample

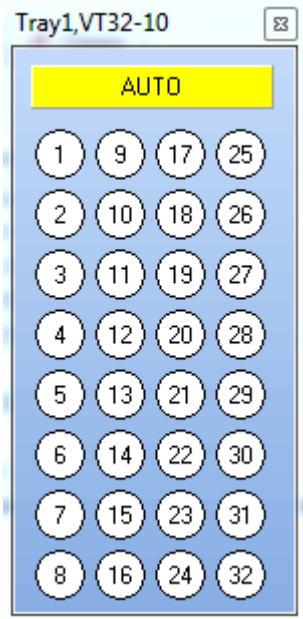
add 300 uL ISTD

add 500 HCl 1N

add 1 mL Hexane/DCM

mix for 20 mins @ 400 rpm

recover org. phase in GC vial

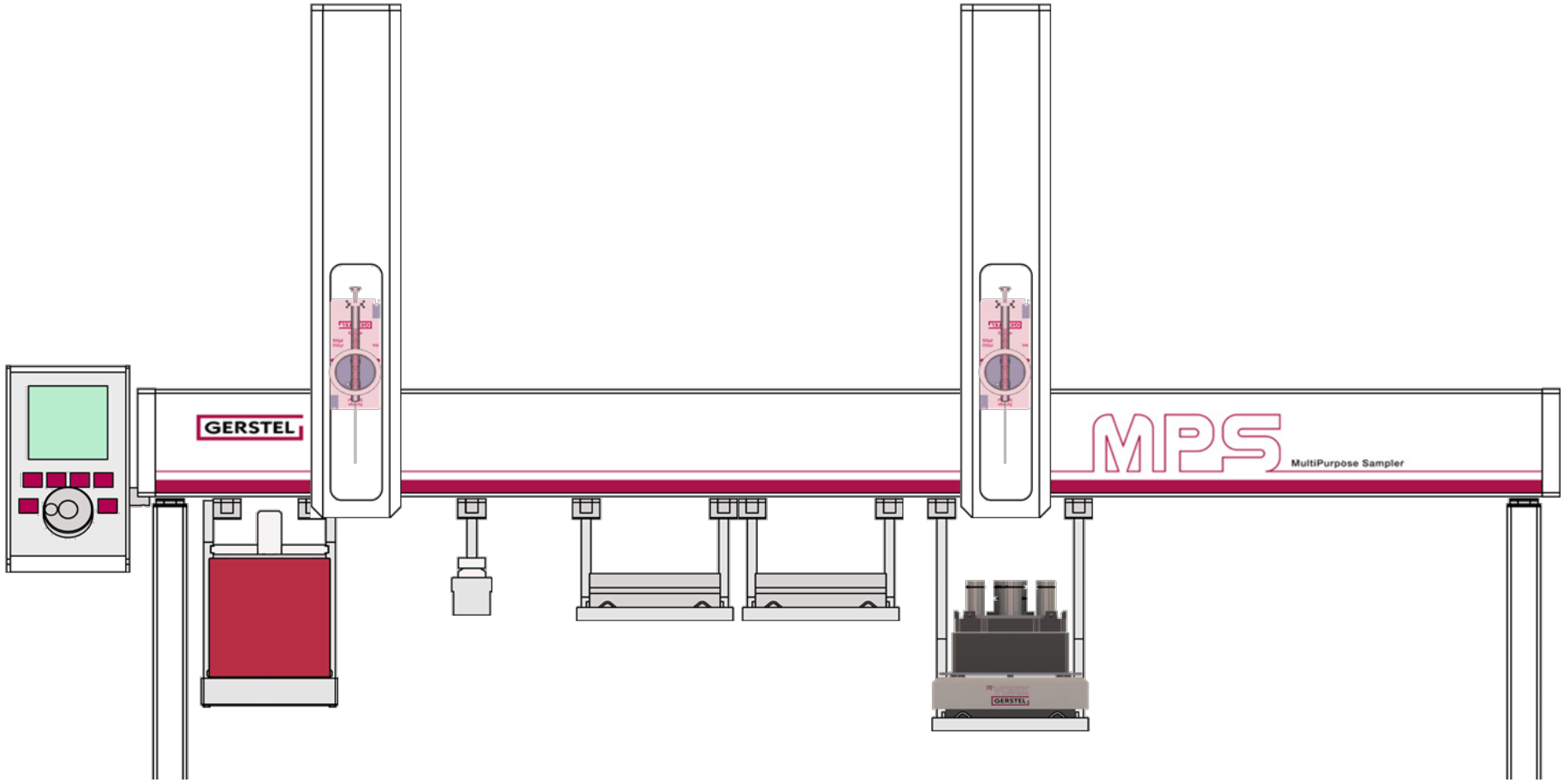


Action	MPS	Method / Value	Source	Vial	Destination	Vial
PREP Vials 1-10		No Overlap				
ADD	Right MPS	Add 5 mL Sample to Extraction Vial	Tray1,VT32-10		Tray1,VT32-10	+16
ADD	Right MPS	Add 300 uL ISTD	SolvRes1		Tray1,VT32-10	+16
ADD	Right MPS	Add 500 uL HCl	SolvRes2		Tray1,VT32-10	+16
ADD	Right MPS	Add 1000 uL DCM/Hexane	SolvRes3		Tray1,VT32-10	+16
MOVE	Right MPS		Tray1,VT32-10	+16	Agitator,AgiTray	
MIX	Right MPS	Mix 20 Min				
MOVE	Right MPS		Agitator,AgiTray		Tray1,VT32-10	+16
WAIT	Right MPS	Wait 3 min				
ADD	Right MPS	Transfer 600 uL @ 27.2 mm to GC Vial	Tray1,VT32-10	+16	Tray2,VT98	
END						

mVorx

- Vortex Mixer
- Capable of vortexing 2, 4 or 10 mL vials
- Top vortex speed of 3000 rpm

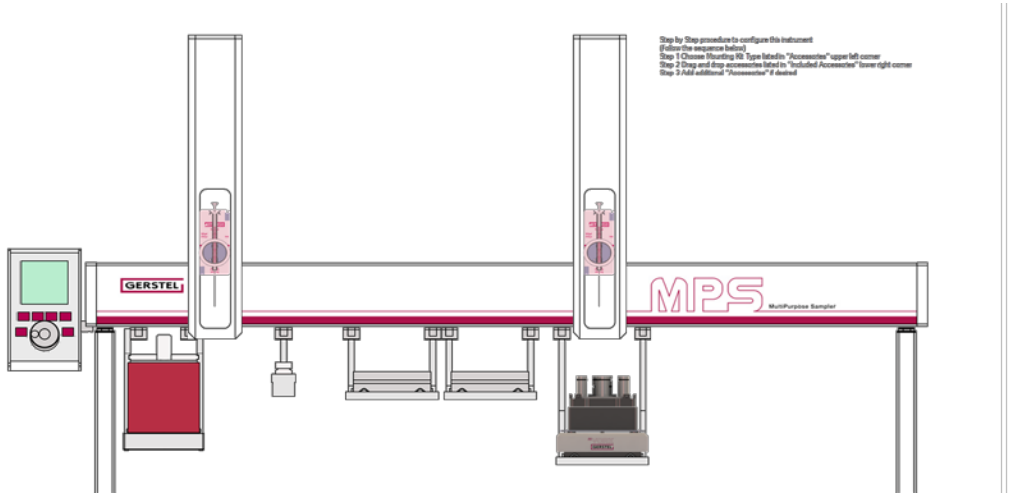
What can we do with the addition of the mVorx ?



What can we do with the addition of the mVorx ?

- More rapid liquid-liquid and liquid-solid extraction

- Vortex mixing enable the break up of soils, food materials enabling more thorough extraction



mVap

- Solvent evaporation station
- Control temperature
  - Range RT-120°C
- Control vacuum
  - Range Atmospheric – 2 mTorr
- Control time
- Fully Automated

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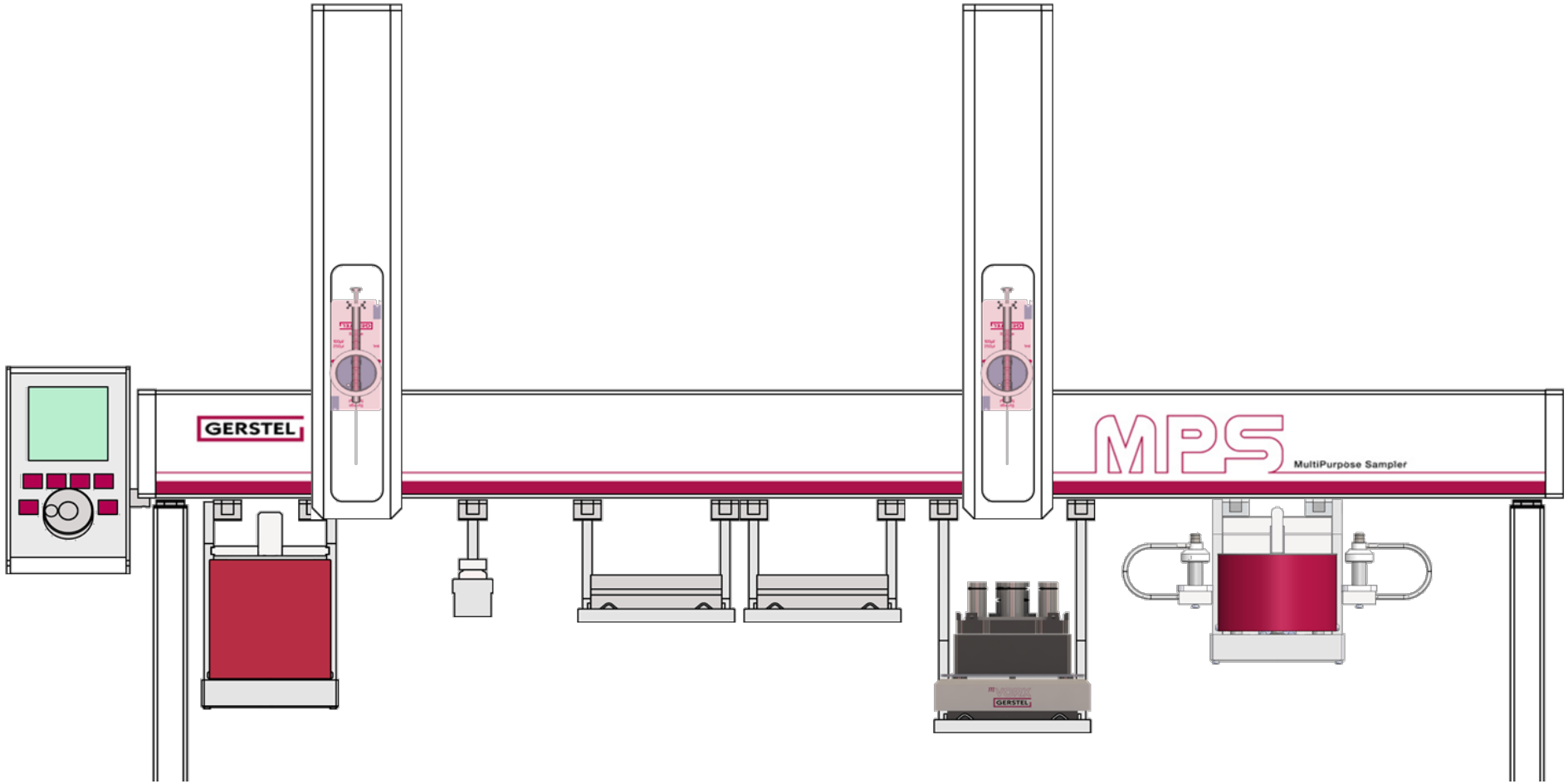
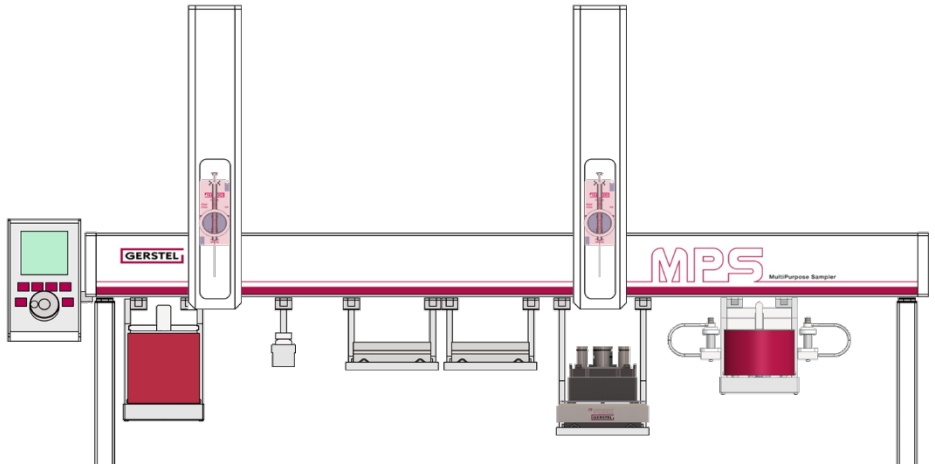


What can we do with the addition of the mVap ?

- Evaporate to dryness
- Solvent exchange
- Use as a second heated agitator for more complex derivatisation procedures
- Use a catcher solvent to evaporate to volume

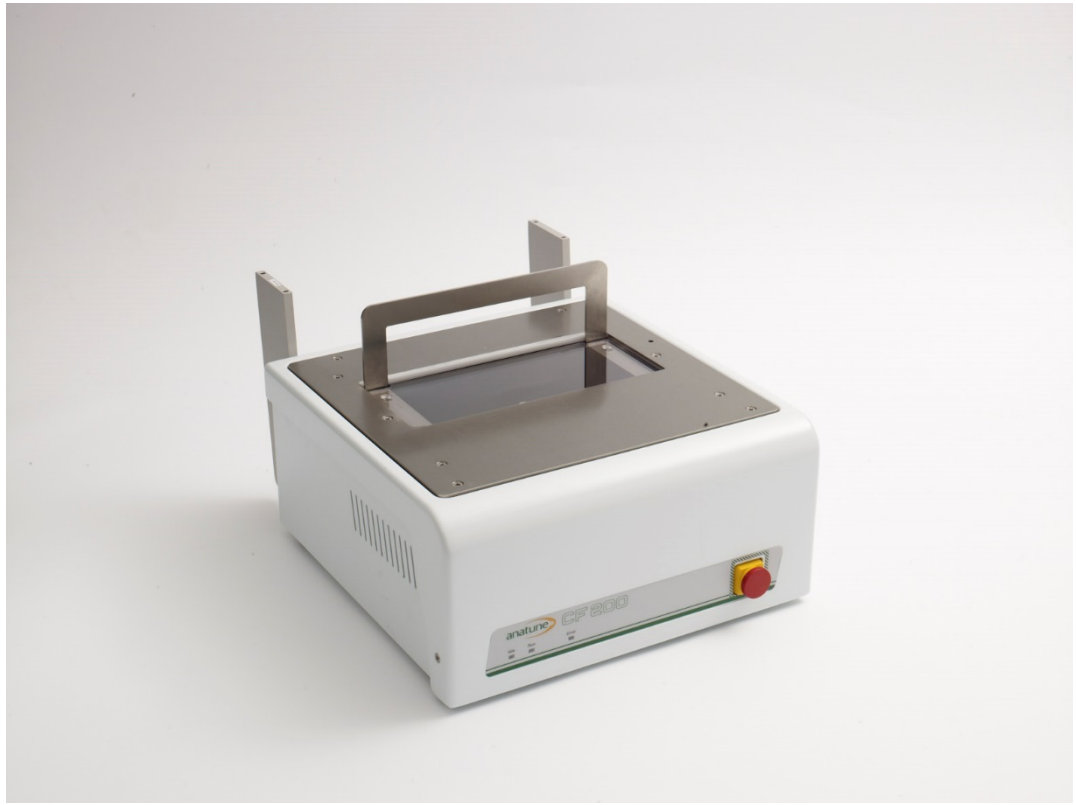


CF-200 Centrifuge

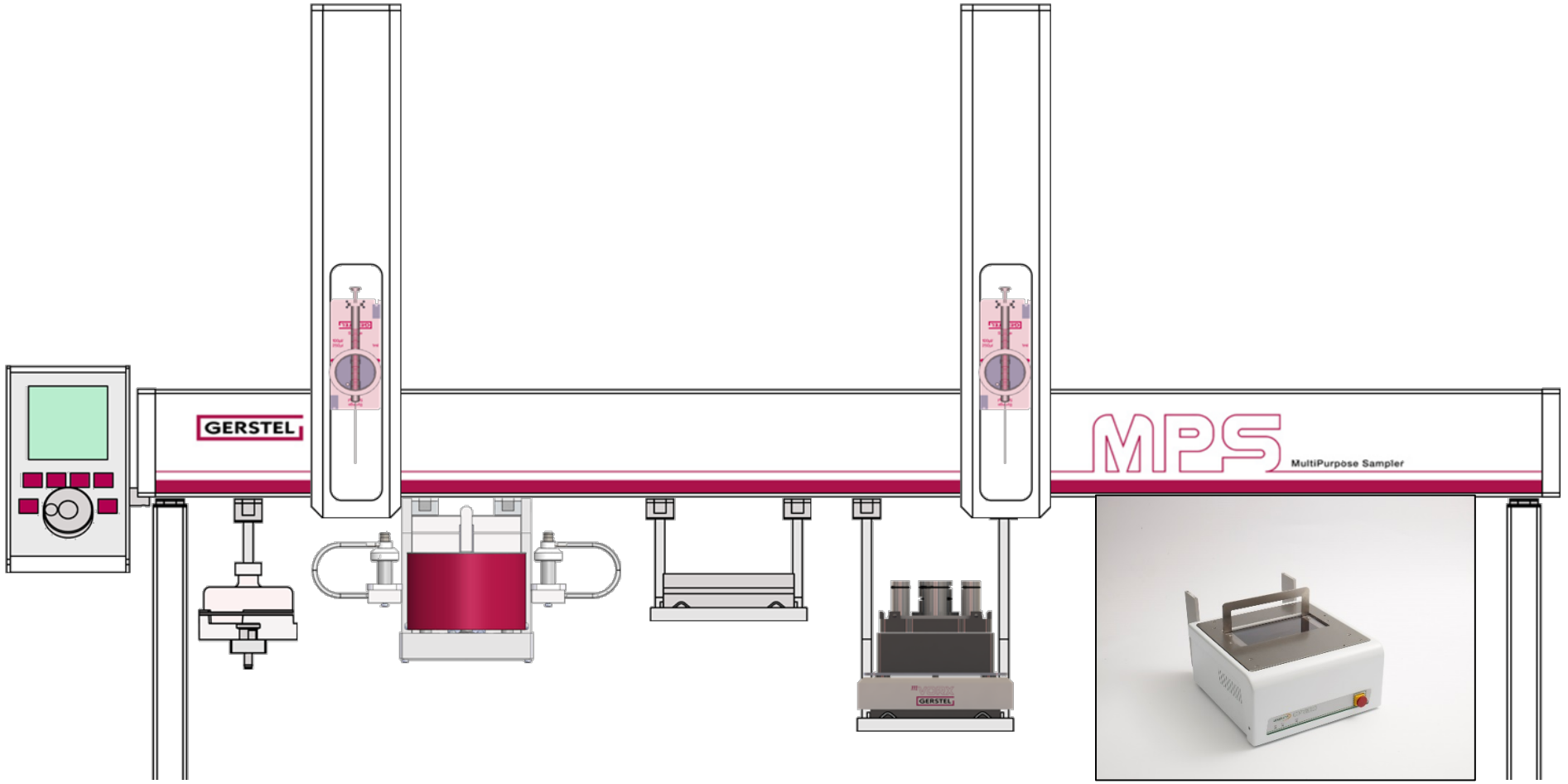
LAUNCHED AT AGILENTS FOOD AND ENVIRONMENTAL MEETING 2016 !!

CF-200

- Capable of centrifuging 2 and 10 mL vials
- Top speed 4500 rpm (2000g)
- 6 positions



Demonstration Kit



Cold Solvent Extraction

- Advantages of automation
  - Once the sample has been weighed no more sample prep required
  - Extracts are all extracted for the same amount of time
  - Reproducibility is better
  - Extracts injected as soon as prepared
- Advantages of LVI
  - Higher sensitivity
  - No need for evaporative techniques

Cold Solvent Extraction

- Weigh 15(±0.5) g of sample into a beaker
- Slowly add 20 g of hexane
- Leave for 90 minutes for osmosis to occur
- Remove hexane layer and concentrate using rotary evaporator
- Inject 1 µL into GC

- Accurately weigh 1.5 g of sample into a 10 mL headspace vial
- Slowly add (100 µL/min) 3.3 mL of hexane (including RTL internal standard)
- Leave for 90 minutes
- Inject 10 µL of hexane layer directly into the GC using large volume injection

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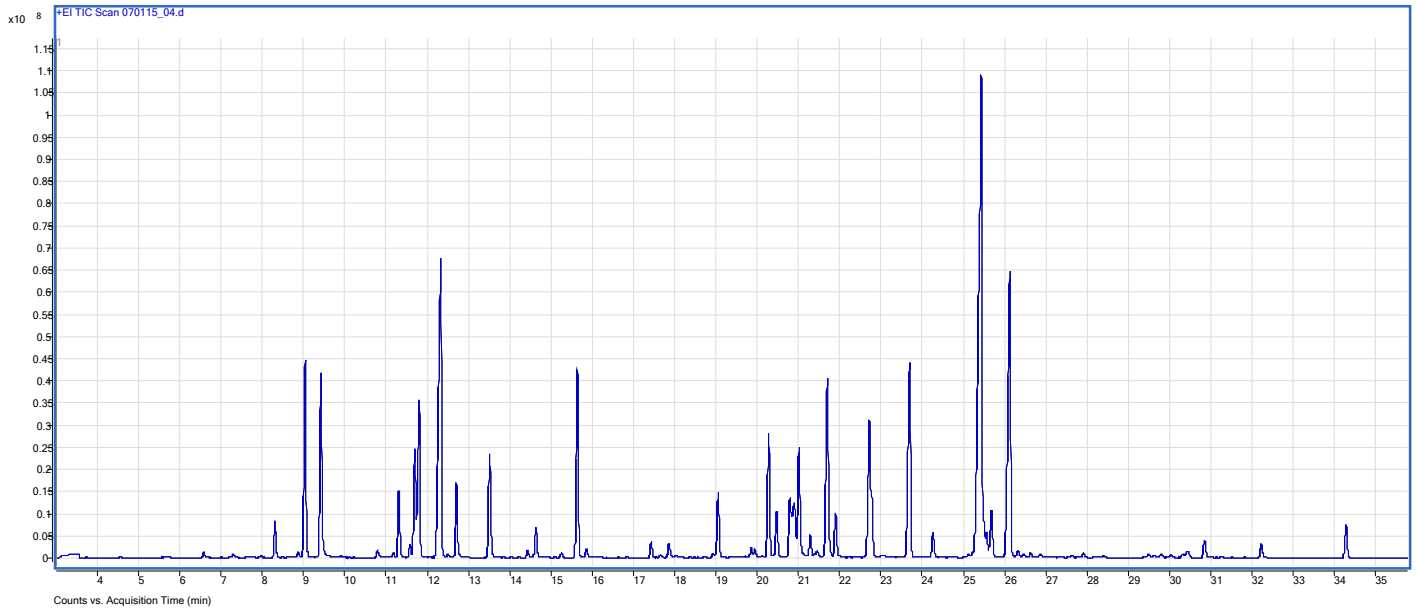
Single extraction of washing liquid



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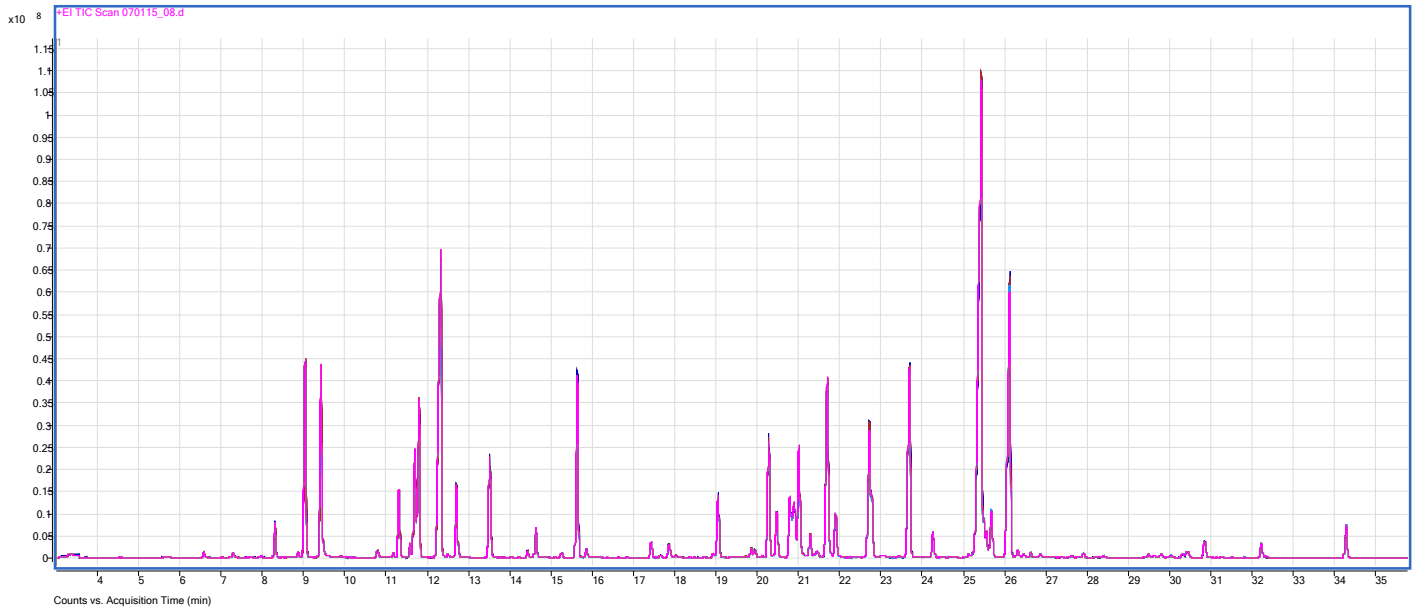
Multiple extractions of washing liquid (n=5)



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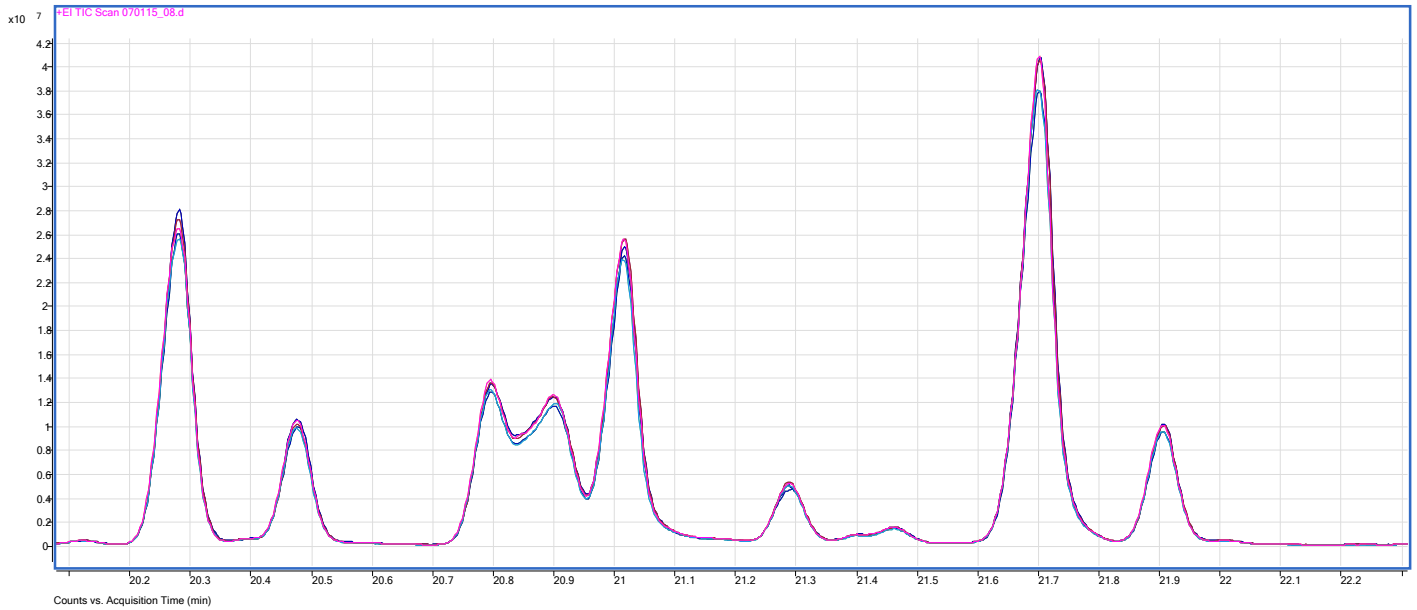
Leave for 90 minutes

Inject 10 µL of hexane layer directly into the GC using large volume injection

Multiple extractions of washing liquid (n=5) Close up



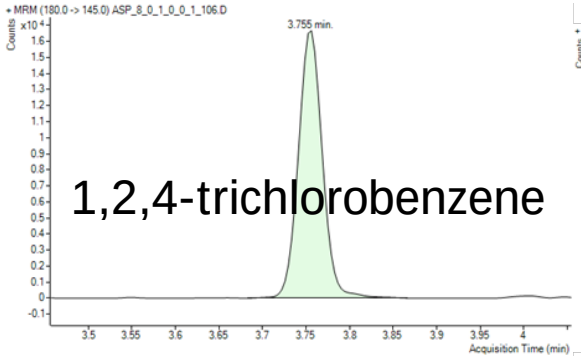
Cold Solvent Extraction

Reproducibility across the chromatogram

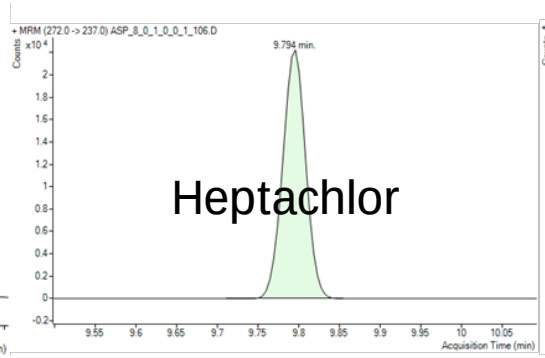
Compound	Cintronellol		α-isomethyl ionone		Tetradecanoic acid	
Run	Retention time	Peak Area	Retention time	Peak Area	Retention time	Peak Area
1	12.7136	56045027	20.2816	97668611	34.2790	29016820
2	12.7108	53148282	20.2816	91880261	34.2845	26519954
3	12.7136	53417358	20.2816	95410824	34.2846	28986281
4	12.7108	52473018	20.2816	92067951	34.2818	29156653
5	12.7108	53495398	20.2788	95516782	34.2789	28465086
Mean	12.7119	53715817	20.2810	94508885	34.2818	28428959
Stdev	0.0015	1362858	0.0013	2484001	0.0028	1099015
%RSD	**0.0121**	**2.54**	**0.00617**	**2.63**	**0.00817**	**3.87**

ALS Coventry

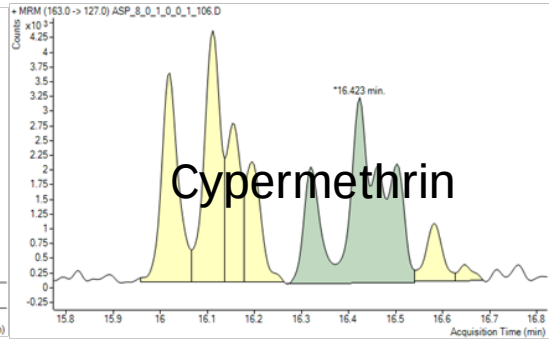
10 ng/L



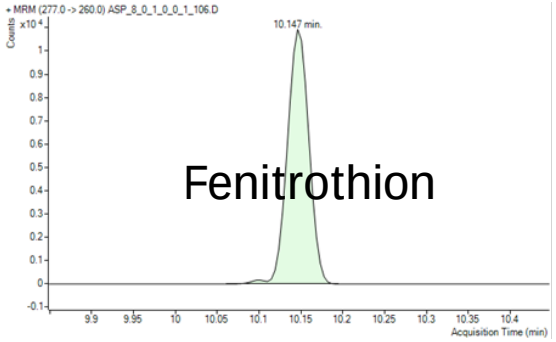
1,2,4-trichlorobenzene



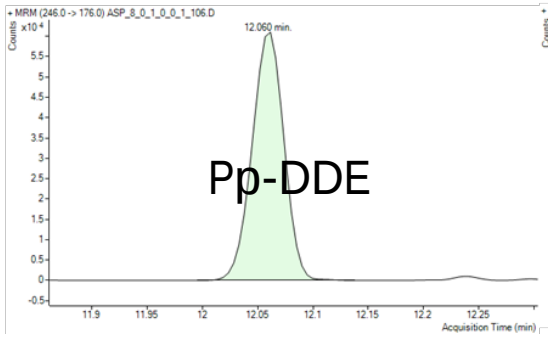
Heptachlor



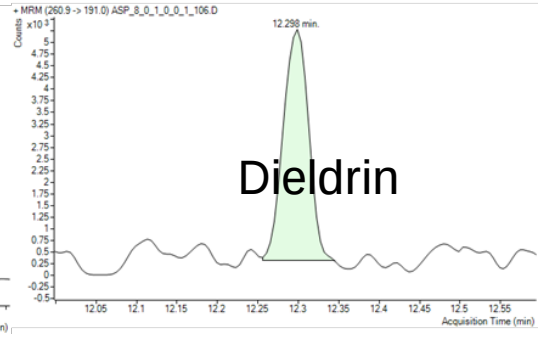
Cypermethrin



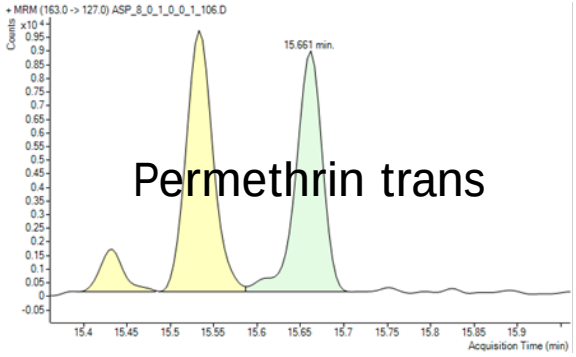
Fenitrothion



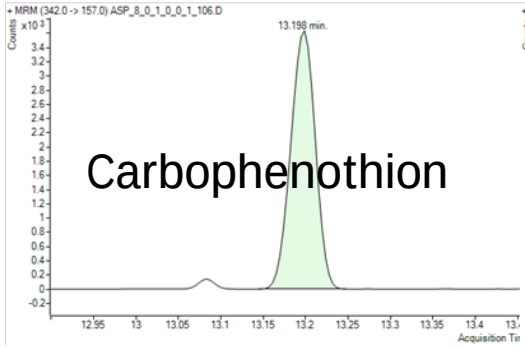
Pp-DDE



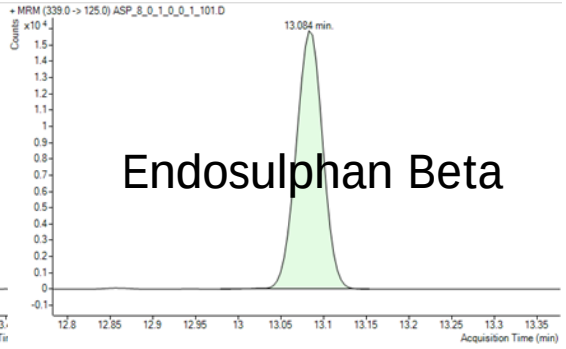
Dieldrin



Permethrin trans



Carbophenothion



Endosulphan Beta

Linearity

Pesticide	Transition	Correlation coefficient (r2)	Fit
124-Trichlorobenzene	180.0 -> 145.0	0.9985	Linear
HCH-beta	181.0 -> 145.0	0.9982	Linear
Dichlobenil	171.0 -> 100.0	0.9998	Linear
Chlordane-#1-trans	372.8 -> 265.9	0.9952	Linear
Tecnazene	261.0 -> 203.0	0.9988	Quadratic
Cyfluthrin	163.0 -> 127.0	0.9987	Quadratic

Reproducibility

	D3 1,2,4 Trichlorobenzene	13C6-HCH-gamma	d14-Trifluralin	d10-Parathion-ethyl
Standard	Area	Area	Area	Area
1	299878	366733	579973	131833
2	313286	379972	580459	123728
3	313128	369943	552668	119763
4	312448	364992	549098	121595
5	309863	360063	559213	126399
6	314287	362141	529283	114059
Standard deviation	5404.3	7102.1	19595.0	6040.4
Mean	310481.7	367307.3	558449.0	122896.2
%RSD	**1.7**	**1.9**	**3.5**	**4.9**

Extraction of Solid Samples

- Weigh 15(±0.5) g of sample into a beaker
- Slowly add 55 (± 0.5) g of hexane
- Leave for 90 minutes for osmosis to occur
- Remove hexane layer and concentrate using rotary evaporator
- Inject 1 µL into GC

- Accurately weigh 1.5 g of sample into a 10 mL headspace vial
- Slowly add (100 µL/min) 8 mL of hexane (including RTL internal standard)
- Leave for 90 minutes
- Inject 10 µL of hexane layer directly into the GC using large volume injection

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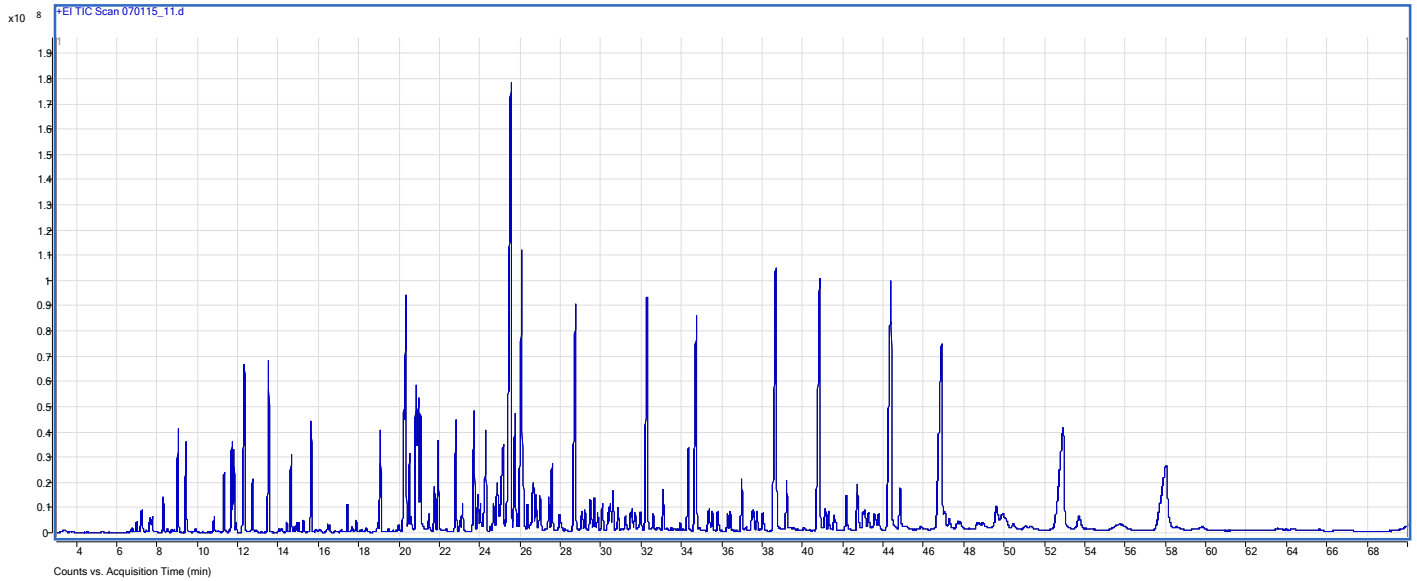


- Weigh approximately 5 mg of oregano
- Add 500 uL of methanolic hydrochloride
- Mix at 70 °C at 500 rpm
- Wait 1 minute
- Add 500 uL of hexane
- Add 500 uL of water
- Wait for 1 minute
- Inject 10 uL of the hexane layer via LVI on the CIS4


x10 6
+EI EIC(74.0366) Scan 1909014_08.D
49.3255
53.1902
56.7341
45.0127
20.3730
x10 6
+EI EIC(74.0366) Scan 1909014_09.D
49.3209
53.1857
56.7295
45.0148
20.3751
x10 6
+EI EIC(74.0366) Scan 1909014_10.D
49.3218
53.1866
56.7304
45.0157
20.3759
12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60
Counts vs. Acquisition Time (min)

FAME	%CV (n=6)
Methyl tetradecanoate	6.9
Methyl hexadecanoate	4.7
Methyl octadecanoate	6.2
Methyl eicosanoate	5.8
Methyl docosanoate	3.9

The Beast of Norwich

The Beast of Norwich

- Start with freeze dried plant material
- A saponification
- A liquid-liquid extraction
- Evaporated of each extracts to dryness
- Derivatisation of the contents of the vial to give the final analysis solution

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

Summary

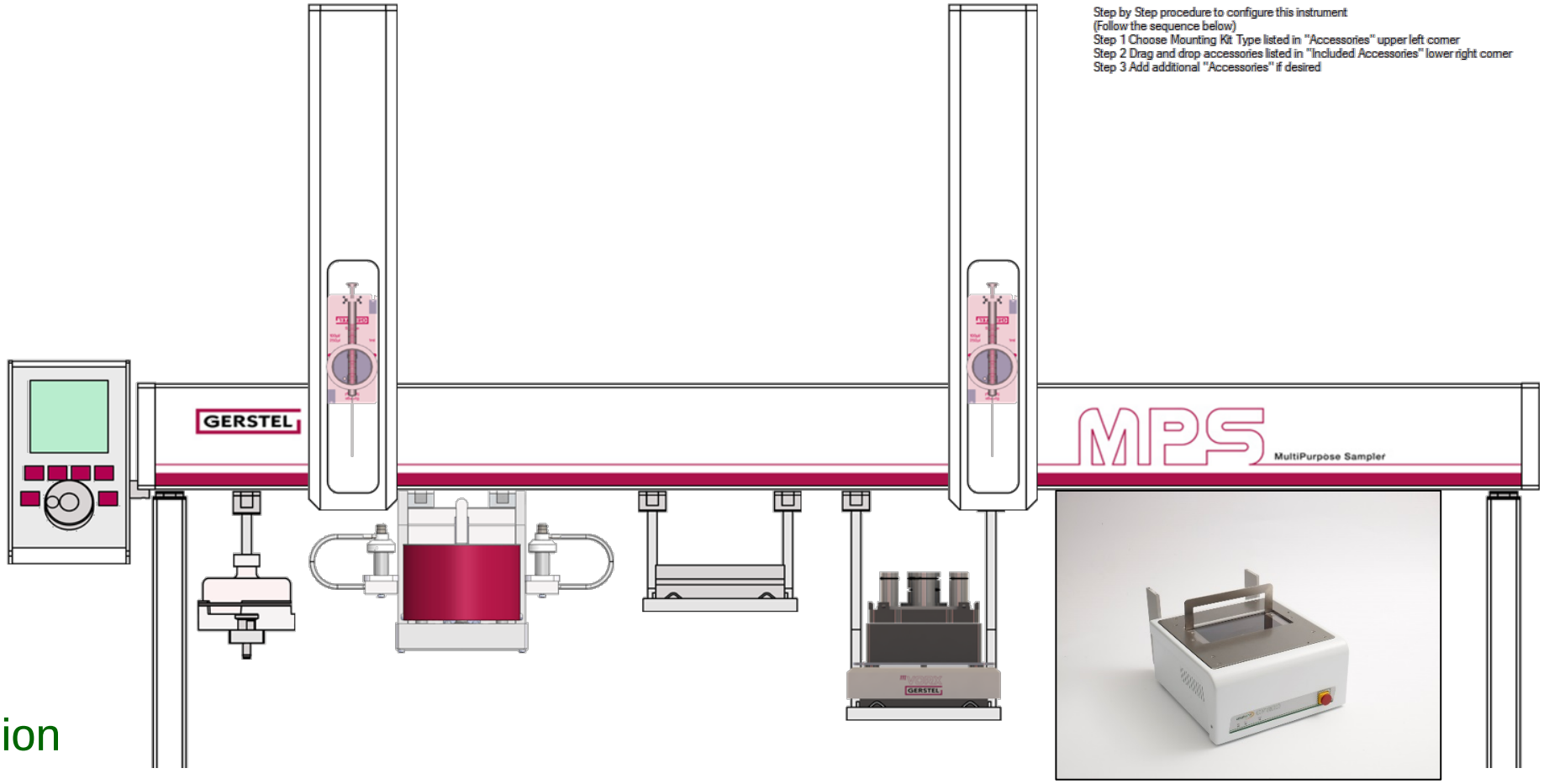
- Automation of liquid extraction, derivatisation and other simple sample preparation is easily done using an GERSTEL MPS system
- Maestro software is fully integratable with Agilent's Chemstation and Masshunter software
- Automation can save you solvent and analyst time and make the lab safer
- The mVorx, mVap and the new CF200 centrifuge create a flexible system which can be applied to a number of techniques

Acknowledgements

anatune

Thank You

Any Questions ?



Liquid-solid extraction

METHANOLIC EXTRACTION OF SOILS