Use of automated sample preparation techniques with GC-SQ, QQQ, and QTOF for aqueous samples

Dan Carrier, Applications Laboratory Manager
Anatune

www.anatune.co.uk

Purpose of my presentation

" To show how we can automate challenging applications – distilled spirits"

Summary of Presentation

- **Introduction**
  - Anatune

- **Automated (established) sample preparation techniques**
  - ITSP (Instrument Top Sample Preparation)
  - Twister (SBSE)
  - ATEX (Automatic Tube Exchange) and QTOF
  - DHS

Anatune

- Girton, Cambridge (March 2012)

- VAR for Agilent
  - GC and LC products
  - MSD, QqQ, QTOF
- Gerstel
  - MPS – DHS, Twister, ITSP
- Focus - Sell and Support Solutions
  - Wide number of industries - Environmental, Food and Flavours, Petrochem, Pharmaceutical, Forensic, and Clinical
  - In Applications doubled in size –Team of 4

Workshops

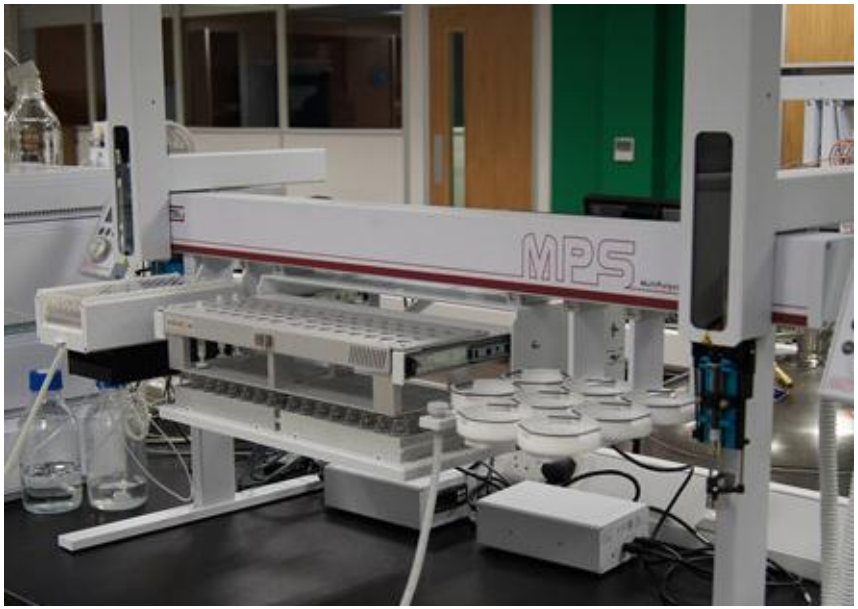
- Twister
- SPME
- MPS training
- NDMA/Metaldehyde
- 1D/2D GC – (distilled spirits)
- GC-QTOF x 2
- GC-QTOF - metabolomics

- Dual Head MPS Solution with LC/UV
  - Formaldehyde and acetaldehyde in air

Right MPS (5 ml Syringe)

Add 5 ml MeCN
10 ml air push

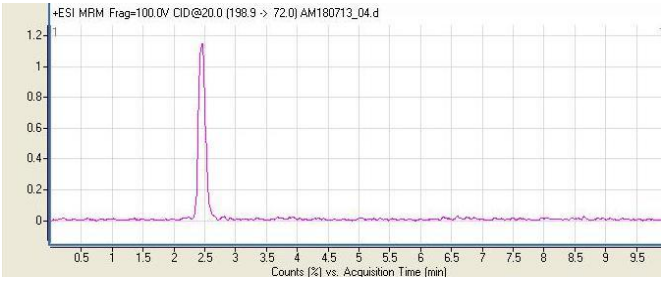
Left MPS (1 ml)
Mix extract
Collect into sealed 1.5 ml HPLC/GC vial (cooled tray)
Load 100 ul onto 10 ul loop



ITSP & LC/MS/MS

- Diurons in water
  - Enrich 10 ml
  - Elute in 0.8 ml

Chromatogram Monuron (quantifier transition) in standard 0.40 µg/L after extraction



Correlation coefficient	Calibration after extraction
Monuron	0.9989
Isoproturon	0.9995
Diuron	0.9997
Linuron	0.9984

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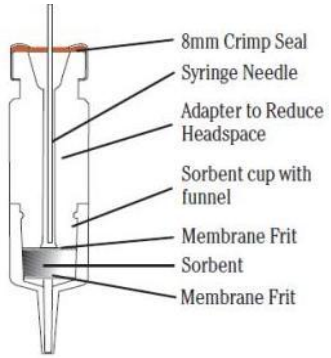
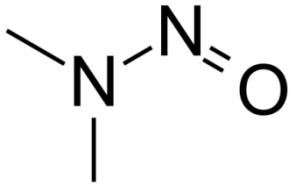
Instrument Top Sample Preparation



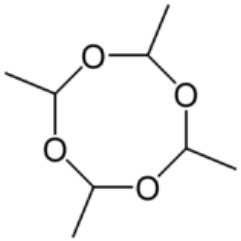
- Small Scale Solid Phase Extraction
- 15-35 mg packing comprehensive range of sorbents (ITSP specials)
- Typical particle size 30-60 micron (100 Amstrong)
- Application – describe their use NDMA and Meltaldehyde - Water industry
- Guys and St Thomas Hospital (over 200 samples per week) LC

Harm - NDMA

- NDMA - Industrial by-product many processes (needs to be <100ng/l in water)



- Metaldehyde Widespread Pesticide (Regulatory limit 100 ng/l in drinking water)



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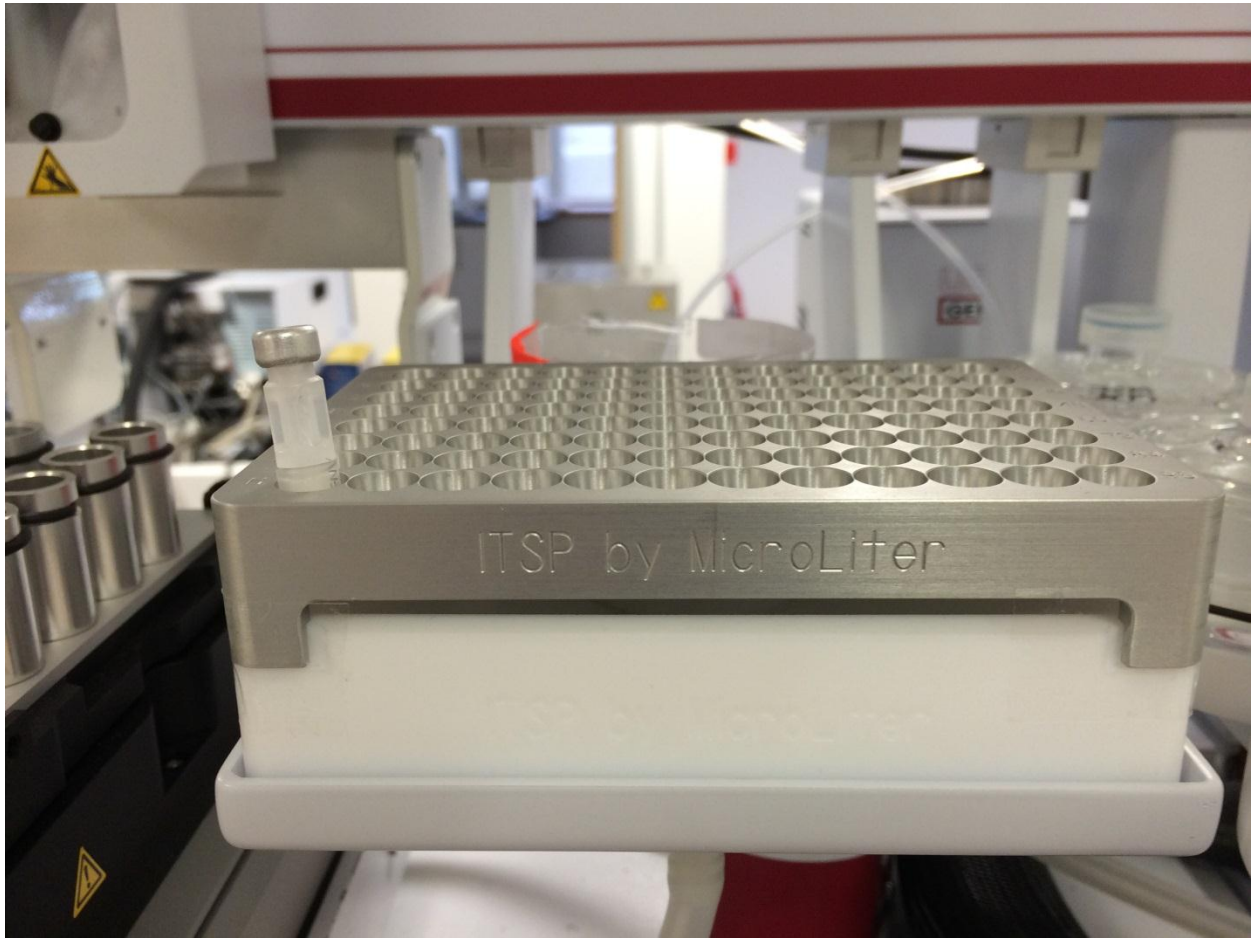
ITSP SOLUTIONS, INC.

Agilent Technologies

GERSTEL

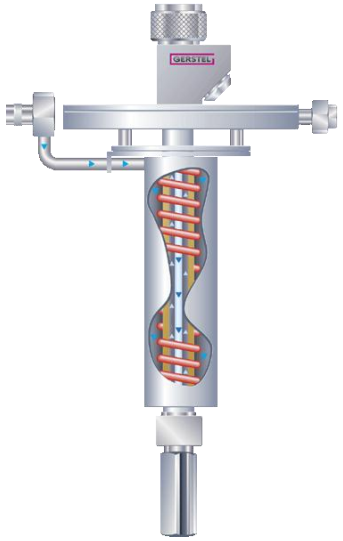
Close up of Tray



Automated Sample Prep

10 ul Syringe

2.5 ml HS Syringe



- Brand this set up - Multiflex
  - Consists of Dual Head MPS
  - Thermal Desorption unit – Cold Inlet System - PTV

Coconut Charcoal ITSP cartridges (NDMA)
ENV (Metaldehyde)

Right MPS (2.5 ml Headspace syringe)

Conditioned 750 µl dichloromethane

1000 µl of methanol

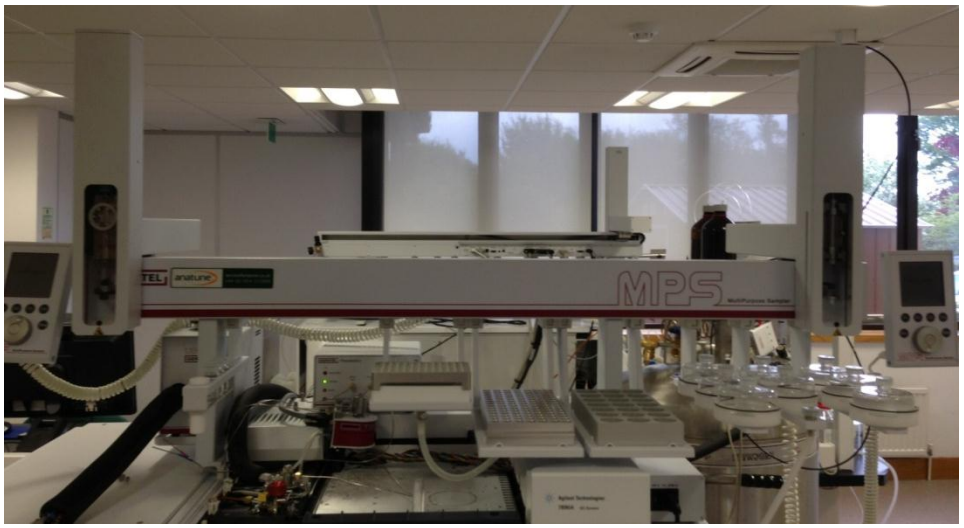
Equilibrated 2000 µl of HPLC grade water

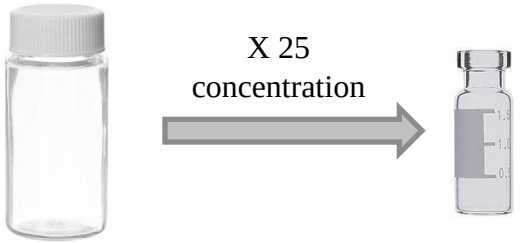
Load 10 ml of sample (in water)

Dried 15 minutes

Eluted 400 ul dichloromethane

Left MPS (10 ul) Large Volume injection

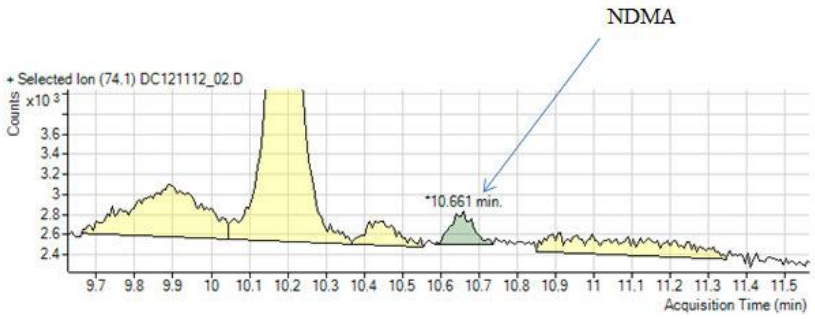




GC/QqQ

- GC/MS triple quad Application
  - Increased Sensitivity and Selectivity

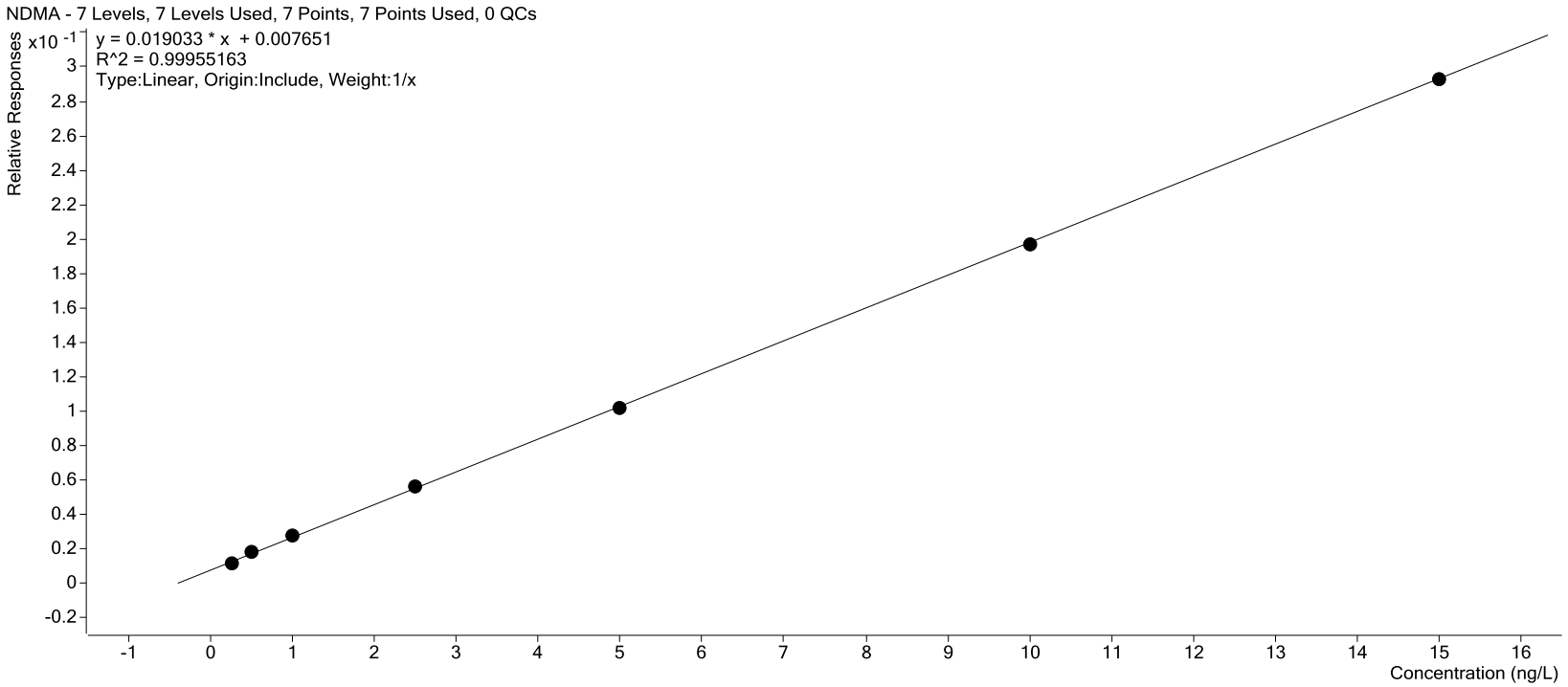
Direct comparison at NDMA at 0.125 ng/ml (without extraction)



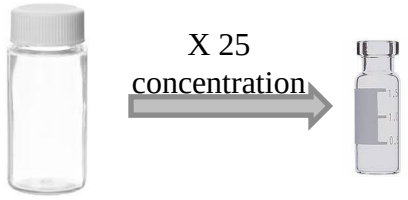
Single Ion Monitoring

Multiple Reaction monitoring

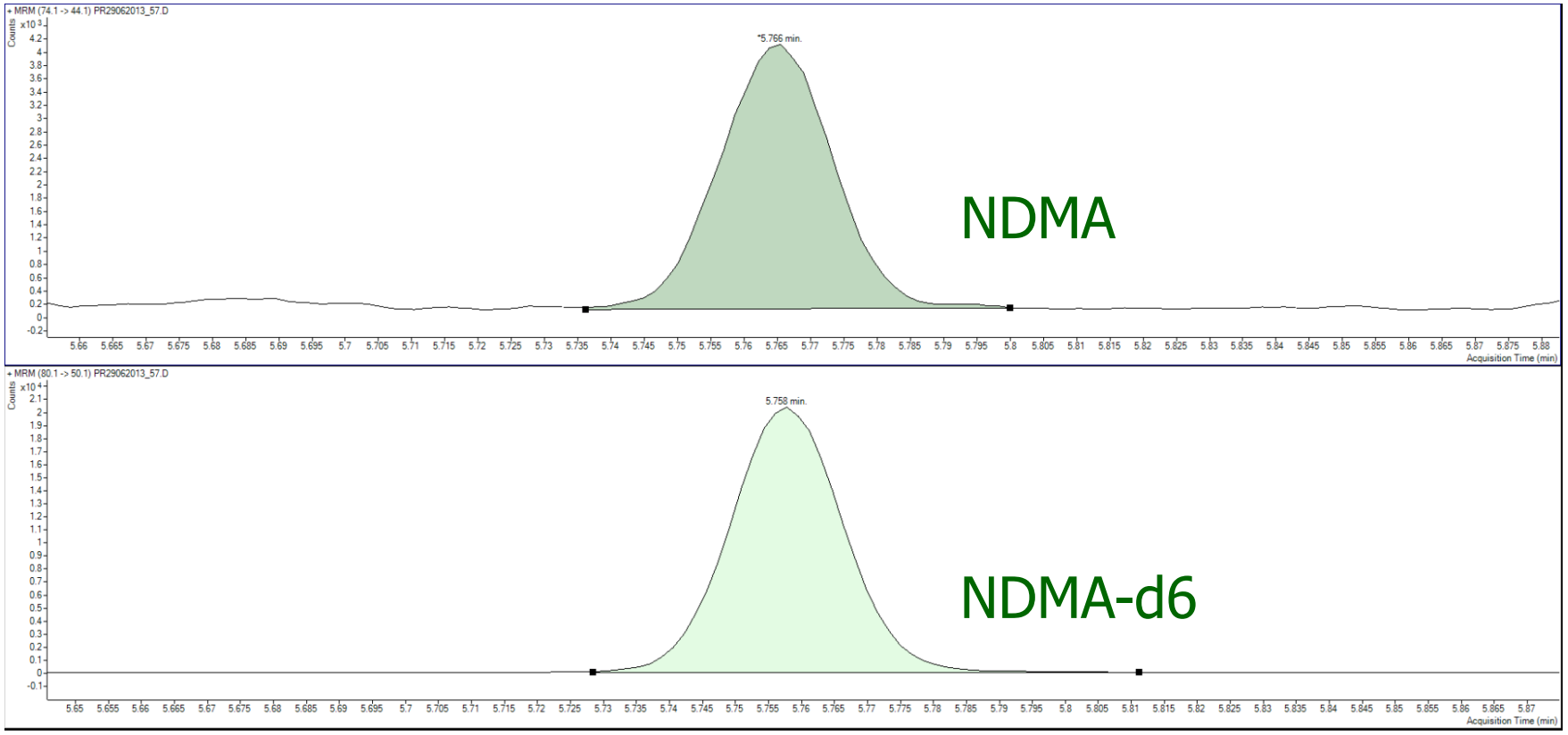
Extracted Water - NDMA



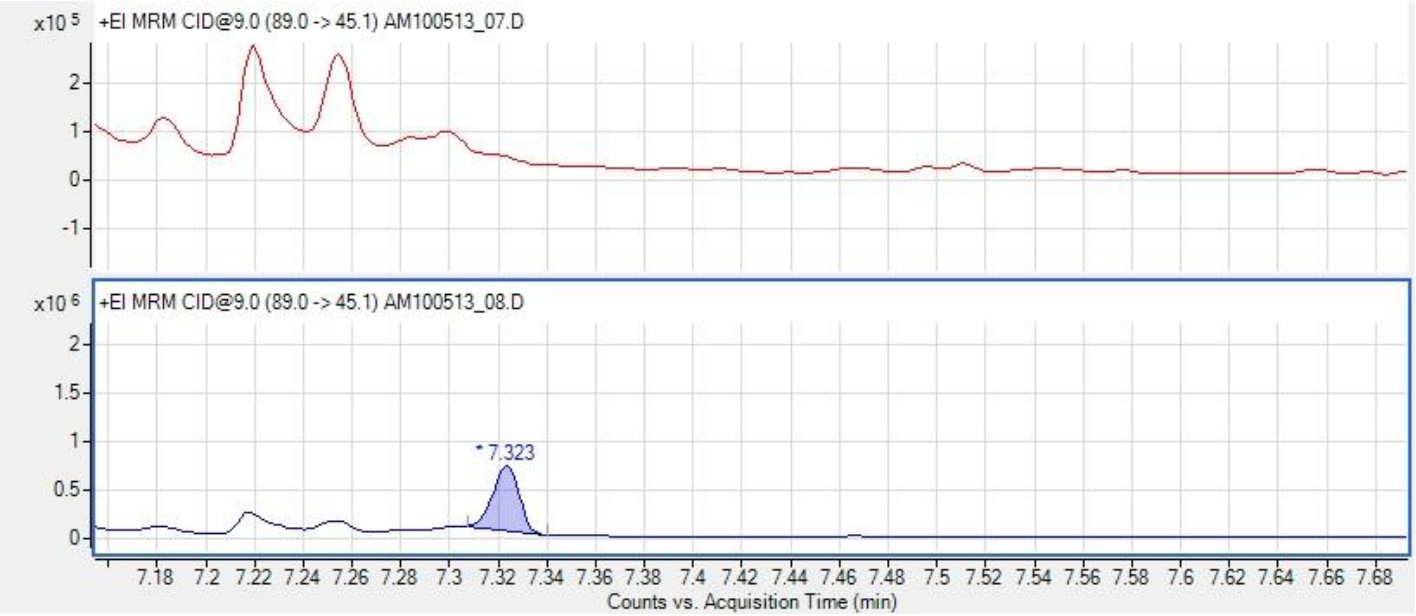
Water spiked to build seven point calibration from 0.25 to 15 ng/l.
Correlation Coefficient of 0.9995.



Standard Extracted Water 10 ng/l

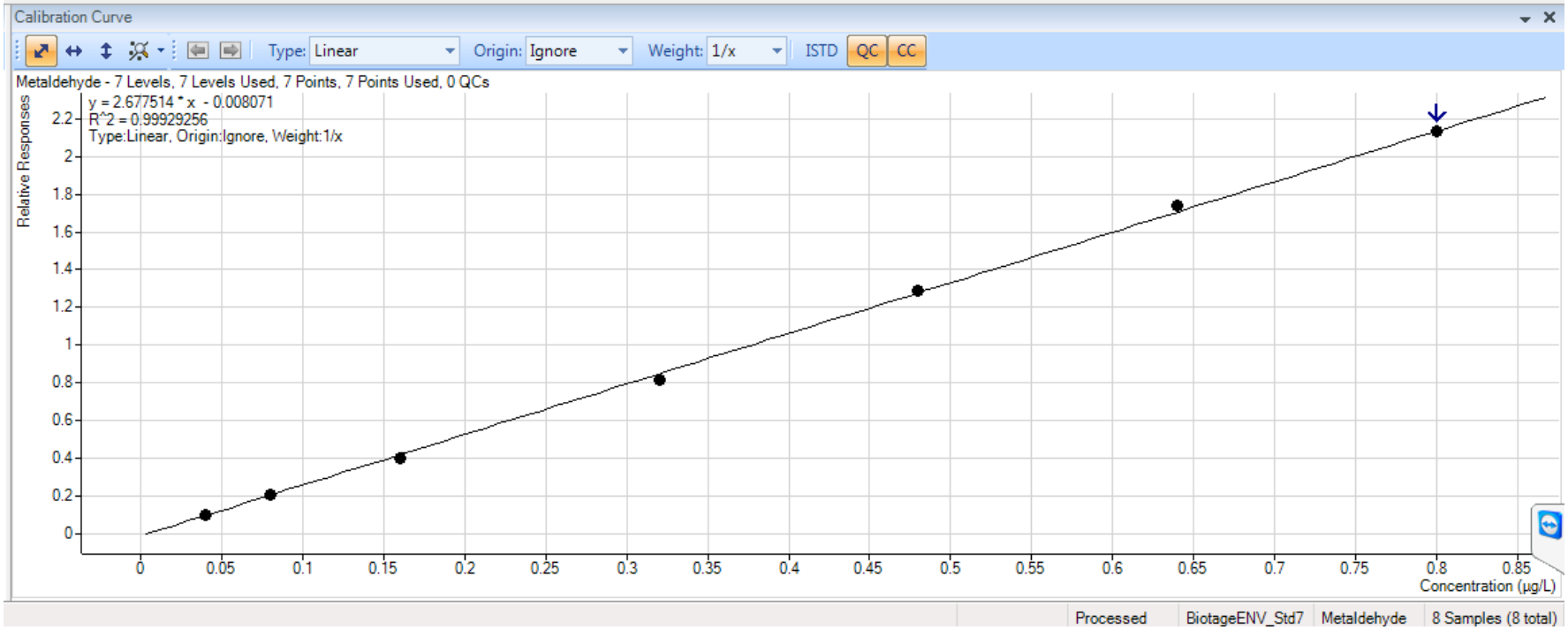


40 ng/l Metaldehyde extracted standard comparison with blank



Detection limit approx 2 ng/l (based on signal to noise from this standard)

Extracted Water - Metaldehyde



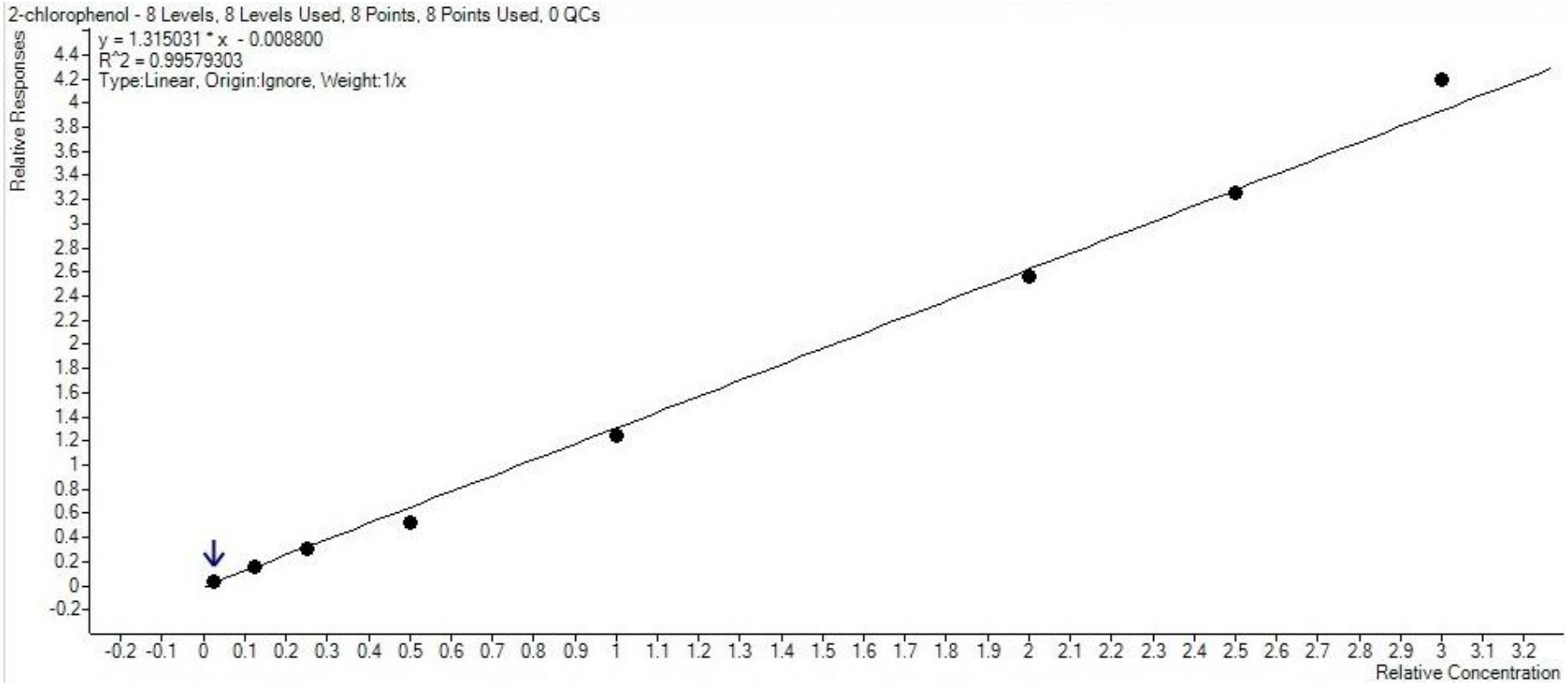
Water spiked to build seven point calibration from 40 to 800 ng/l.
Correlation coefficient of 0.9993.

Recovery and precision of water extractions at 60 and 700 ng/L (Metaldehyde)

Amount spiked (µg/L)	0.06080	0.70400
Amount detected (µg/L)	0.05734	0.63858
	0.05721	0.71908
	0.06000	0.70449
	0.05628	0.72256
	0.05641	0.72204
Mean	0.05745	0.70135
SD	0.0015	0.035856
% RSD	2.61	5.11
% Recovery	**94.49**	**99.62**

Severn Trent Water (STW) Bridgend - Collaboration

- ITSP for Taste and Odour – prove concept
- Developed QQQ methods number of T&O compounds
- Range 1 ng/l to 120 ng/l



Linearity 8 point calibration 1ng/l- 120 ng/l

R^2= 0.995 for 2-chlorophenol
R^2= 0.995 for 2-methylphenol
R^2= 0.992 for 2,4-dichlorophenol
R^2= 0.992 for 2,3-dichlorophenol

	2-chlorophenol	2-methylphenol	2,4-dichlorophenol	2,3-dichlorophenol
Amount spiked (ng/ml)	0.0300	0.0300	0.0300	0.0300
Amount detected (ng/ml)	0.0344	0.0323	0.0313	0.0330
	0.0340	0.0349	0.0255	0.0287
	0.0328	0.0334	0.0282	0.0291
	0.0352	0.0355	0.0306	0.0321
	0.0302	0.0315	0.0320	0.0342
	0.0313	0.0308	0.0311	0.0339
Mean	0.0330	0.0331	0.0298	0.0319
SD	0.0019	0.0019	0.0025	0.0024
% CV	5.78	5.69	8.25	7.44
% Recovery	109.94	110.23	99.31	106.19

Phases currently available

Stock Number	Sorbent Vendor	Vendor Part #	Bedmass	Sorbent	Substrate	End-Capping	Separation Mode	Functional Group
07-BABN10-20A	Biotage	9600-0010	10	EVOLUTE ABN	Polymeric		Mixed (RP+SCX)	C18 + BSA
07-BAX10-20A	Biotage	9603-0010	10	EVOLUTE AX	Polymeric		Mixed (RP+SAX)	RP + QA
07-BC810-20A	Biotage	9290-0025	10	ISOLUTE C8	Silica	Endcapped	Reverse	C8
07-C1810-20A	Orochem	OCC18	10	Orpheus C18	Silica	Endcapped	Reverse	C18
07-C1820-20A	Orochem	OCC18	20	Orpheus C18	Silica	Endcapped	Reverse	C18
07-C1830-20A	Orochem	OCC18	30	Orpheus C18	Silica	Endcapped	Reverse	C18
07-C810-20A	Orochem	OCC8	10	Orpheus C8	Silica	Endcapped	Reverse	C8
07-C830-20A	Orochem	OCC8	30	Orpheus C8	Silica	Endcapped	Reverse	C8
07-CBA10-20A	Orochem	OCCB	10	Orpheus CBA	Silica		WCX	CBA
07-CN10-20A	Orochem	OCCN	10	Orpheus CN	Silica		Normal	CN
07-DEAP10-20A	Orochem	OCCD	10	Orpheus DEAP	Silica		WAX	DEAP
07-JDVB10-20A	Jordi	40092	10	Gel DVB	Polymeric		Reverse	DVB
07-JNEU08-20A	Jordi	47042	8	Gel eXtreme	Polymeric		Normal + Reverse	Polyamide
07-JSAX10-20A	Jordi	40652	10	Gel SAX	Polymeric		SAX	QA
07-JSCX12-20A	Jordi	41212	12	Gel SCX	Polymeric		SCX	BSA
07-JWAX10-20A	Jordi	41012	10	Gel WAX	Polymeric		WAX	Polyamino
07-JWCX10-20A	Jordi	45002	10	Gel WCX	Polymeric		WCX	CBA
07-NH210-20A	Orochem	OCNH	10	Orpheus Amino	Silica		Normal + WAX	NH2
07-OPSA25-20A	Orochem	OCPSA	25	Orpheus PSA	Silica		Normal + WAX	PSA
07-PH10-20A	Orochem	OCPH	10	Orpheus Phenyl	Silica		Reverse	Phenyl
07-SAX10-20A	Orochem	OCSAX	10	Orpheus SAX	Silica		SAX	QA
07-SCX10-20A	Orochem	OSSCX	10	Orpheus SCX	Silica		SCX	BSA
07-SIL10-20A	Orochem	OCSI	10	Orpheus Silica	Silica		Normal	Silica
07-UAQAX10-20A	UCT	CAQAX1	10	Selectrasorb QA w/ACI AX	Silica		SAX	QA + Acetate Counter Ion
07-UAX10-20A	UCT	CUQAX2	10	Selectrasorb QA + C8	Silica		Mixed (RP+SAX)	C8 + QA
07-UBCXP10-20A	UCT	SSBCX	10	Styre Screen BCX	Polymeric		SCX	BSA
07-UC1810-20A	UCT	CEC18	10	Selectrasorb C18	Silica	Endcapped	Reverse	C18
07-UC18U10-20A	UCT	CUC18	10	Selectrasorb C18	Silica		Reverse	C18
07-UC810-20A	UCT	CUC08	10	Selectrasorb C8	Silica		Reverse	C8
07-UCOCC10-20A	UCT	CHARCOAL21	10	Coconut Charcoal	Other		Special	Carbon

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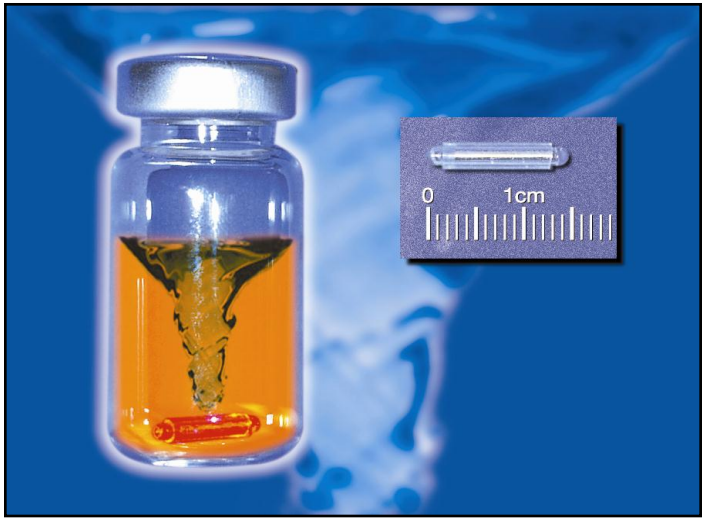
Twister (Stir Bar Sorbtive Extraction)

Theory of Twister SBSE

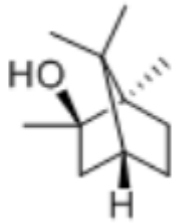
Recovery of analytes onto twister

- How well the analyte can adsorb onto PDMS phase?
- Depend on hydrophobic and lipophilic characteristics of analyte
- Use calculated and theoretical Log K o/w
- PDMS behaves similarly to Octanol

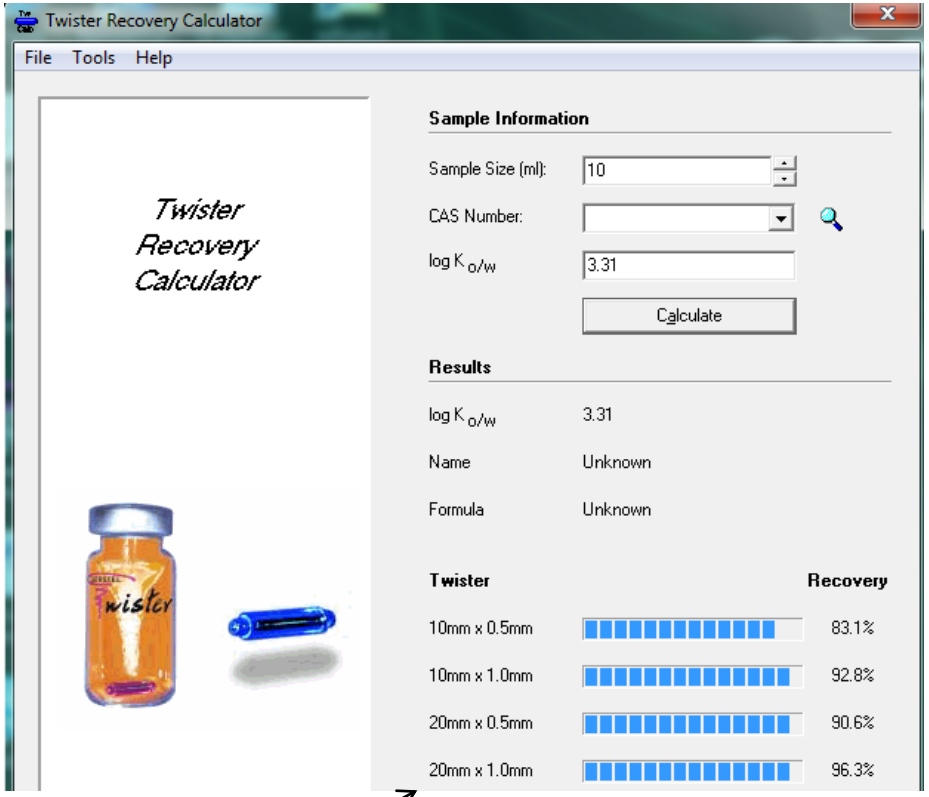


1. Theory of Twister SBSE

- Few examples (Methylisoborneol)



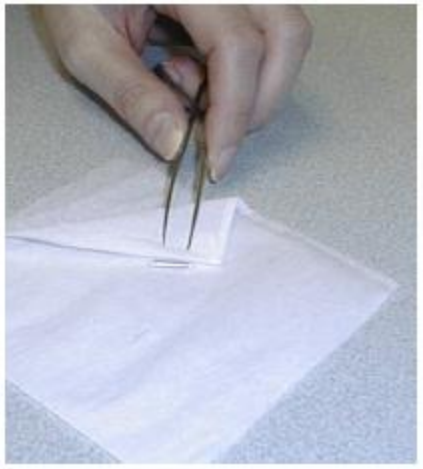
- Log K o/w = 3.31



Take 20mm x 1.0 mm id PDMS twister for comparison

- After required amount of water (10-100 ml) added
  - Each Twister added and placed onto magnetic stirrer plate
  - Left for 2 hours to ensure good recovery and simply leave

- Handling
  - After stirring for 2 hours
  - Remove with magnetic fish
  - Flush with few ml of deionised water
  - Wipe with a tissue
  - Insert into TDU Tube



Twister Set up

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ITSP SOLUTIONS, INC.

TDU

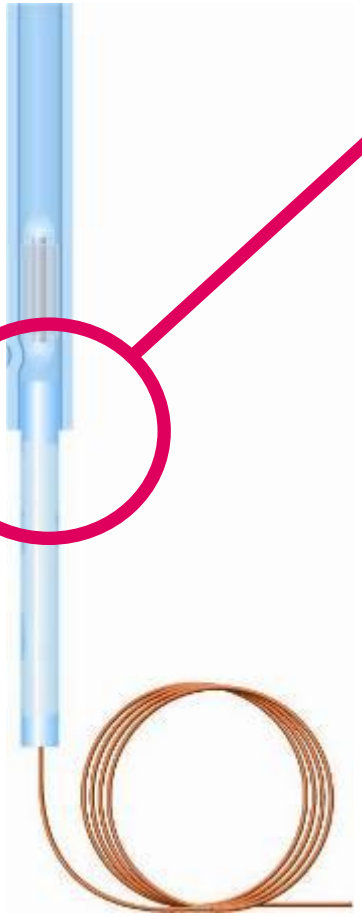
Agilent Technologies

GERSTEL

No Transferline

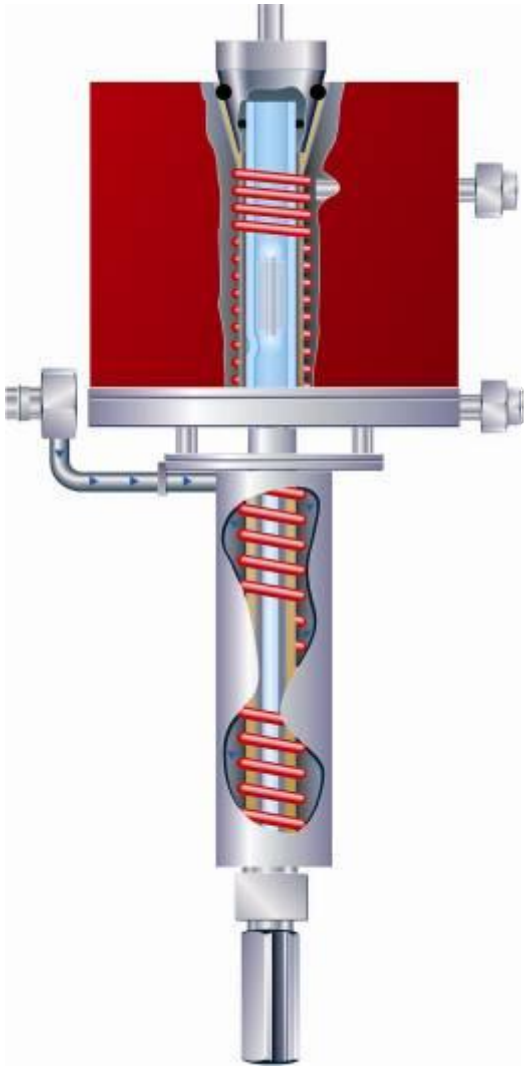
Twister liner

CIS liner



TDU

CIS



Huge concentration effect

Can be over 1000 fold increase in concentration

Twister Applications

- Malodours in Water (Enriching analytes from 10 ml water onto EG twisters 2 cm x 0.5 mm thickness)

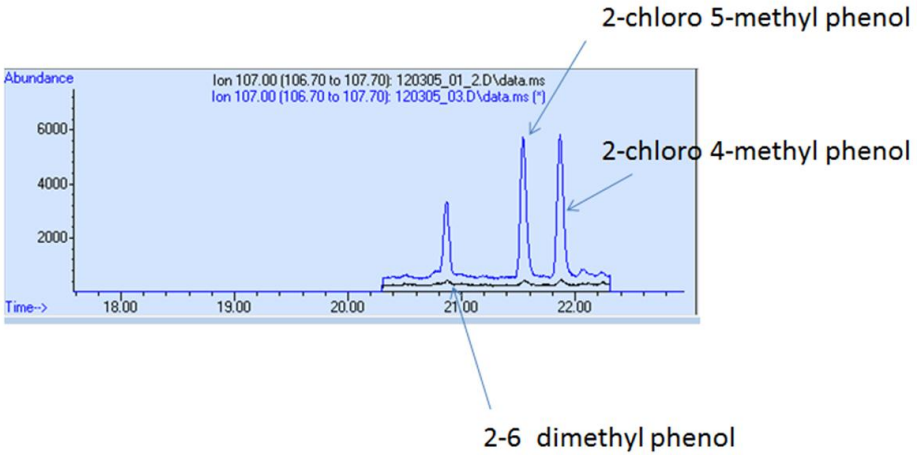
Analyte	% RSD
2-methylphenol	8.7
2-isobutyl-3-methoxypyrazine	5.7
2-chloroanisole	2.7
2-chlorophenol	3.6
2,6 dimethylphenol	5.2
2-chloro-5-methylphenol	4.0
2-bromophenol	6.1
2,3,4-trichloroanisole	1.7
2,4,6-tribromophenol	2.3
2,5-dimethylphenol	3.1

Table 2 Precision achieved for five replicate twister extractions at 0.02 ng/ml.

Twister Applications

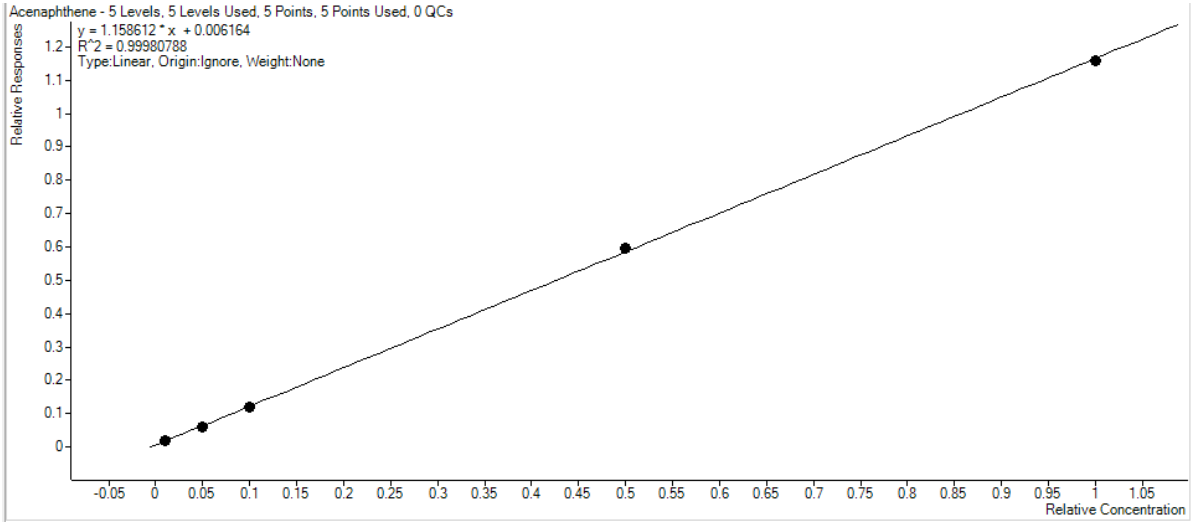
Analyte	Linear Regression (R^2)
2-methylphenol	0.989
2-isobutyl-3-methoxypyrazine	0.991
2-chloroanisole	0.991
2-chlorophenol	0.993
2,6 dimethylphenol	0.991
2-chloro-5-methylphenol	0.991
2-bromophenol	0.992
2,3,4-trichloroanisole	0.991
2,4,6-tribromophenol	0.997
2,5-dimethylphenol	0.993

0.02 ug/l Test mixture (upto 2ug/l 6 point)



Twister SBSE – PAH solution

- 100 ml water samples (2 hours)
  - Dried and placed in TDU tubes
  - SIM 16 PAH (0.02 ug/l to 1 ug/l)
  - Acenaphthene 0.999 (1-2%)



Gerstel - Whisky extraction using twister

- 1 hour extraction diluting 1:1

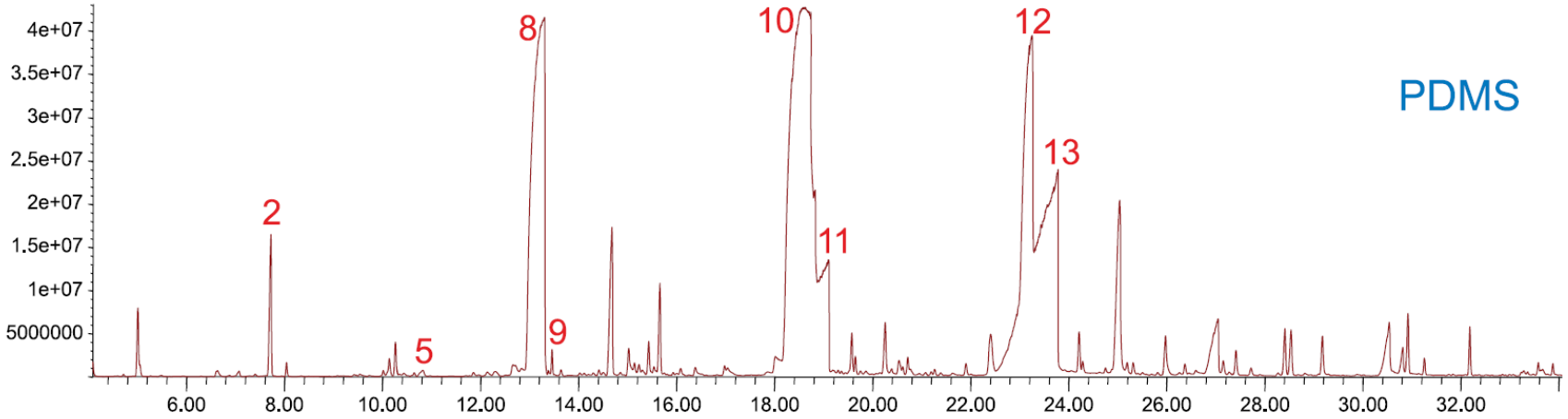


Figure 1. Whisky extraction chromatograms obtained using EG-Silicone, Acrylate and PDMS Twisters, non-polar column separation. 5 mL whisky sample (20 % EtOH (v/v), 1:1 dilution with water), 1000 rpm for 1 hour at room temperature. Peak identification: 1. Phenol; 2. C6 Acid ethyl ester; 3. o-Cresol; 4. p-Cresol; 5. Phenethyl alcohol; 6. o-Ethylphenol; 7. 2,4- Xylenol; 8. C8 Acid ethyl ester; 9. C8 Acid; 10. C10 Acid ethyl ester; 11. C10 Acid; 12. C12 Acid ethyl ester; 13. C12 Acid.

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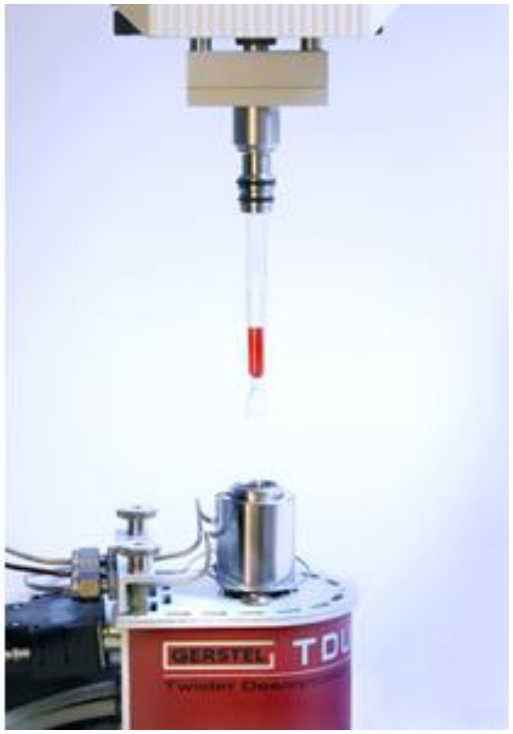
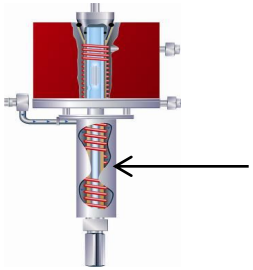
Look at volatiles in an involatile matrix

- Uses same hardware as twister
- Sample is inserted in Microvial (TDU)
- Volatiles desorbed and trapped in CIS
- Non-volatiles (dirty matrix) kept in TDU tube

- Extremely useful to keep liner clean

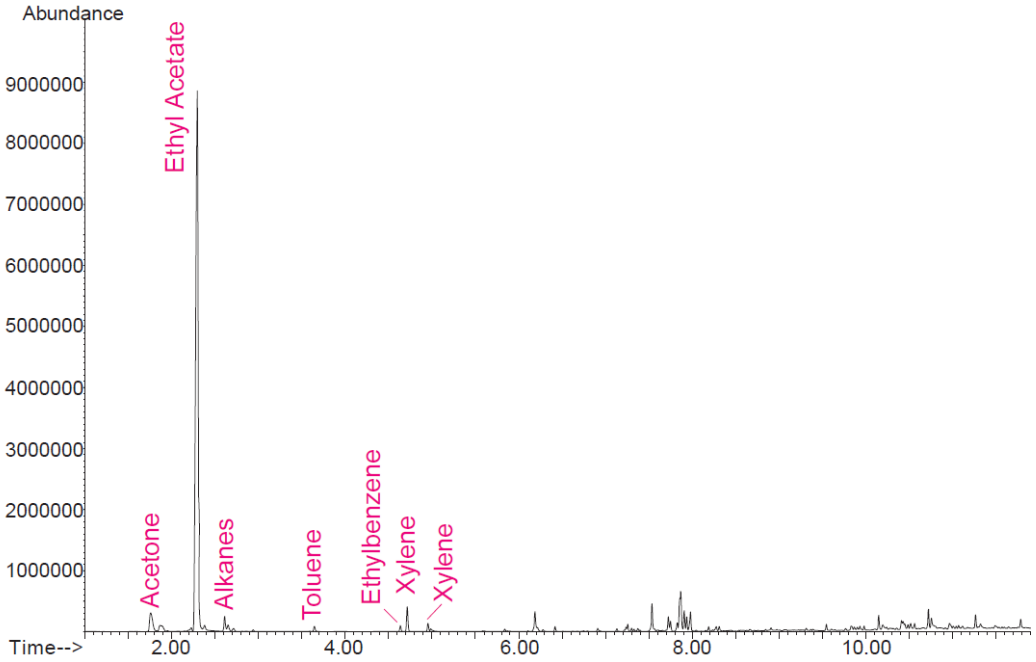
(Dirty non-volatile Matrix or unwanted)





CIS liner kept clean

Direct thermal desorption of volatile analytes in a solid matrix



re 4. GC/MS Total ion chromatogram from thermal extraction of medical tape.

Direct injection – quantify analytes

Septa allows direct injection

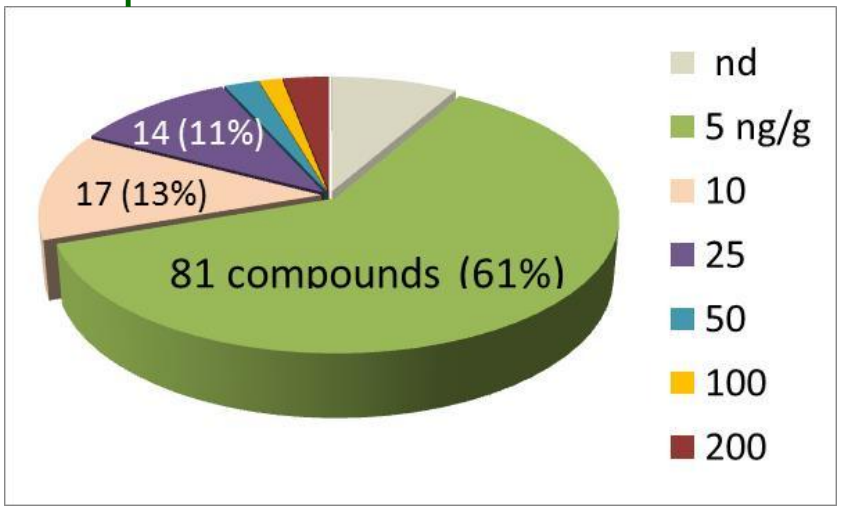
ATEX well suited to GC/QToF

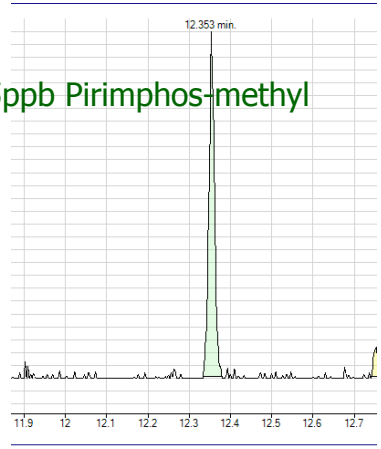
trace analytes in non-volatile matrix

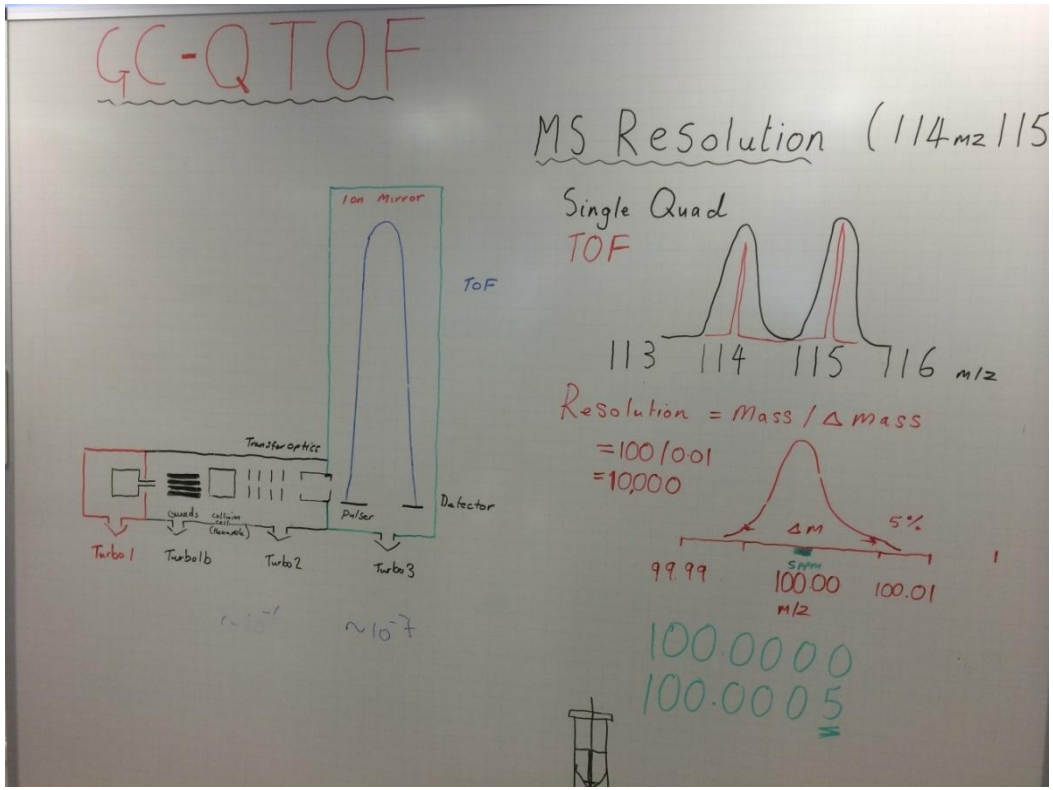
- Highly selective and sensitive Mass spectrometer
- Proof of purchase – FERA GC-QTOF (Richard Fussell)

- Poster at EPRW

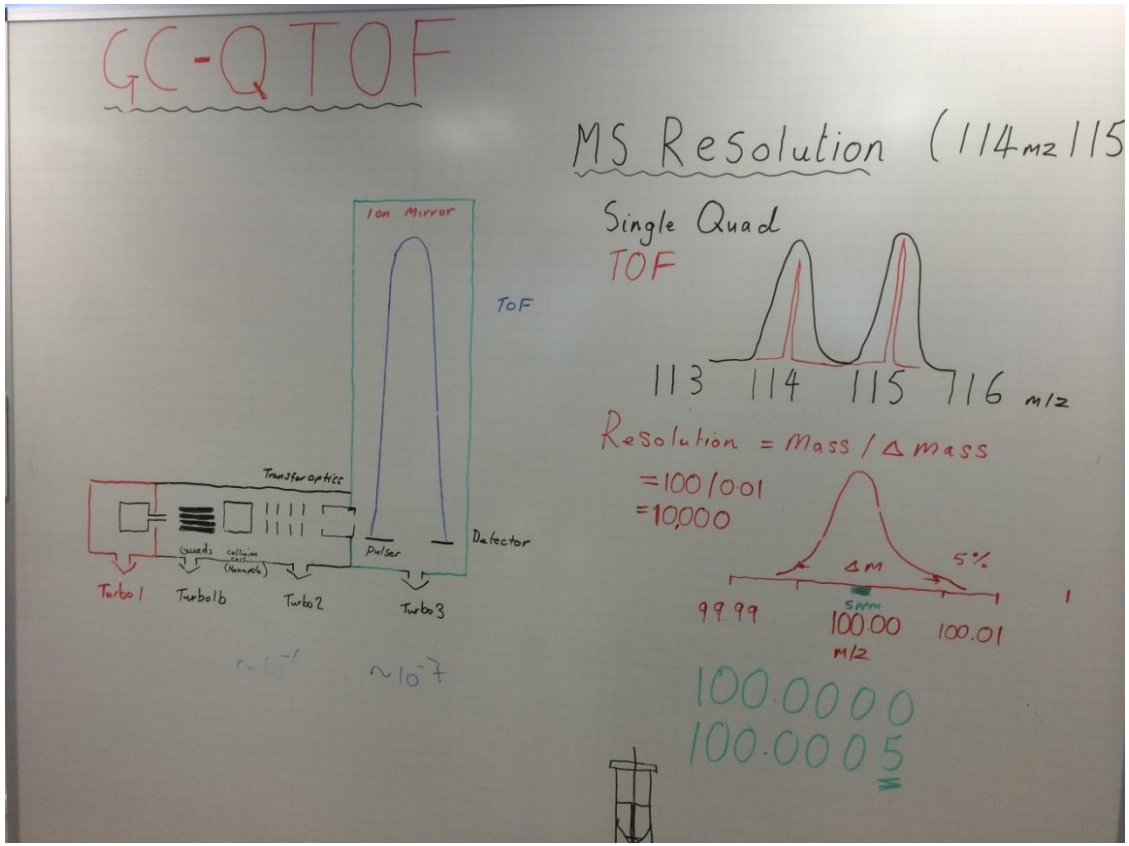
- 132 Spiked Pesticides





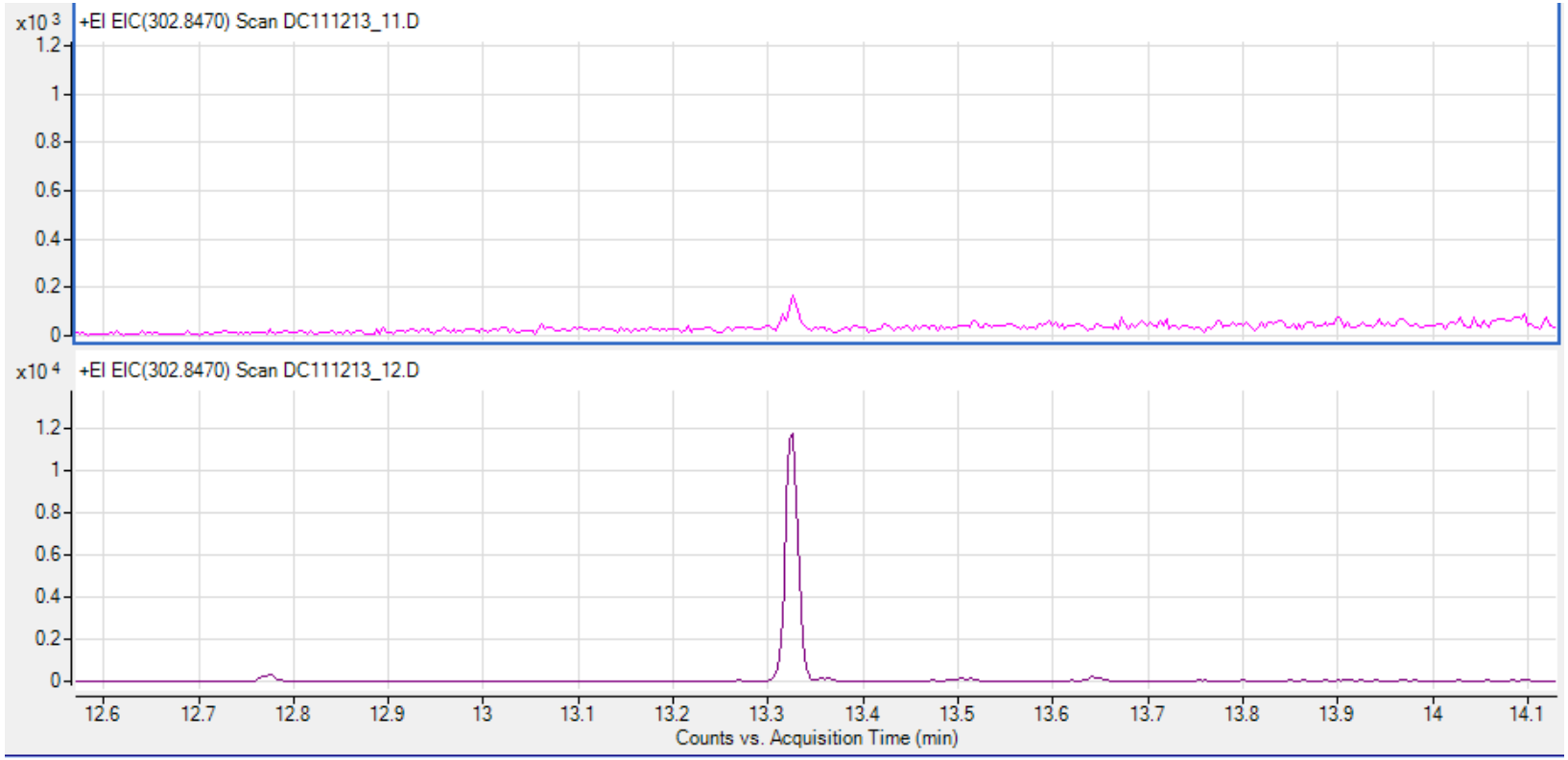


- Consistent Mass Accuracy= (Measured Mass-Theoretical)/ (Theoretical / 1000000) Based on mass 100 m/z
  = (100.0005-100.000)/(100.000 / 1000000)
  = 5 ppm
- Typical MS Resolution High 12000 Low 7000 (0.01 m/z wide)

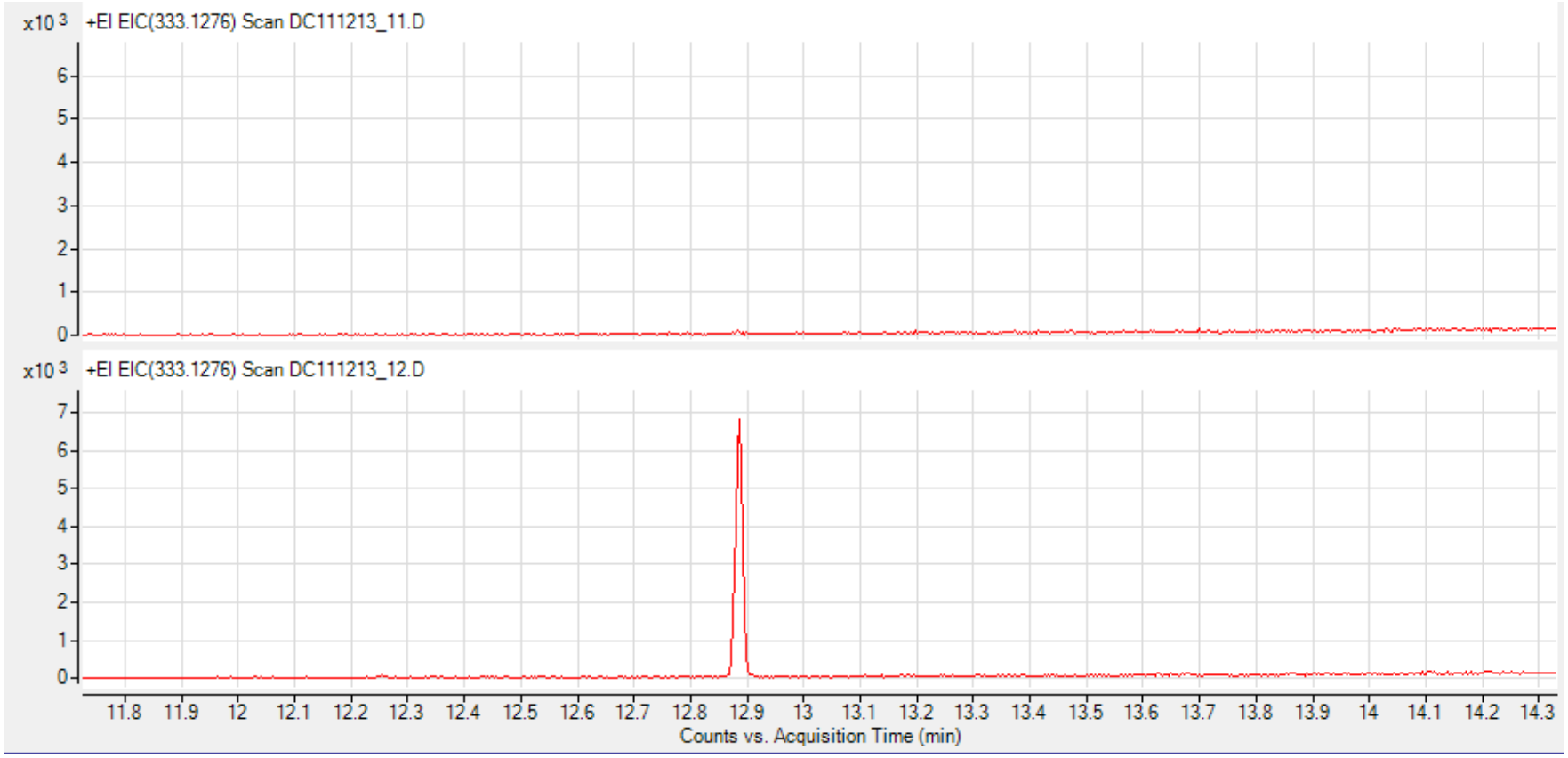


- Structure elucidation – accurate mass
- Filter data with v low mass window to obtain good limits of detection
  - DL approaching MRM QQQ methods

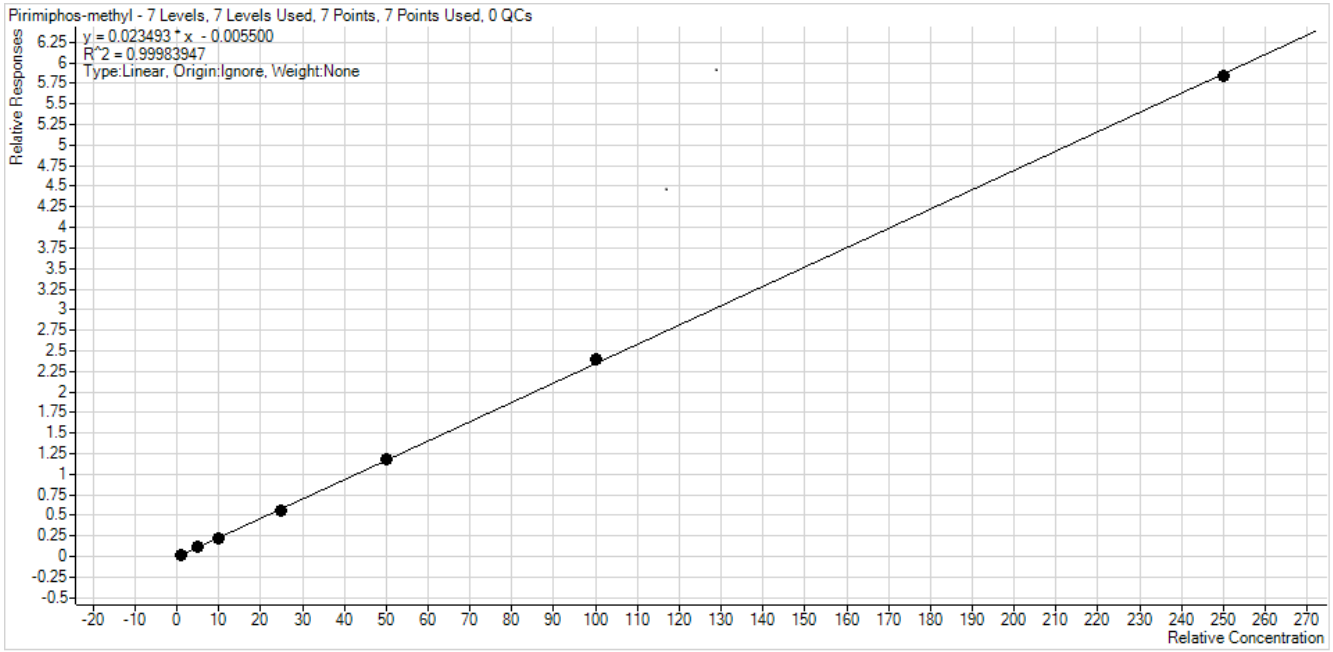
Extracted ion chromatogram of Bromophos-ethyl 10 ng/g in apple extract with 0 ng/g in apple extract



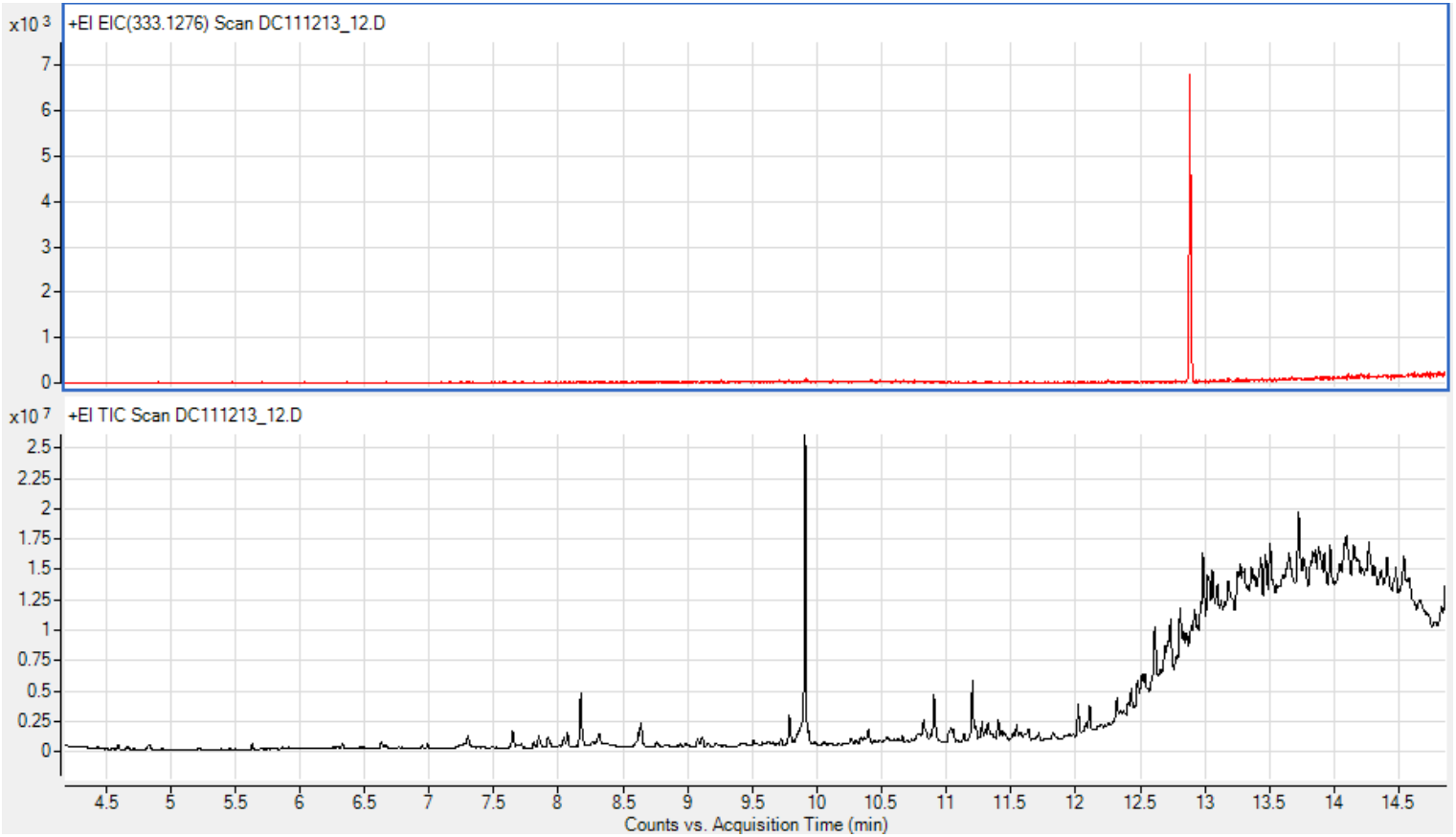
Extracted ion chromatogram of Primiphos-ethyl 10 ng/g in apple extract with 0 ng/g in apple extract



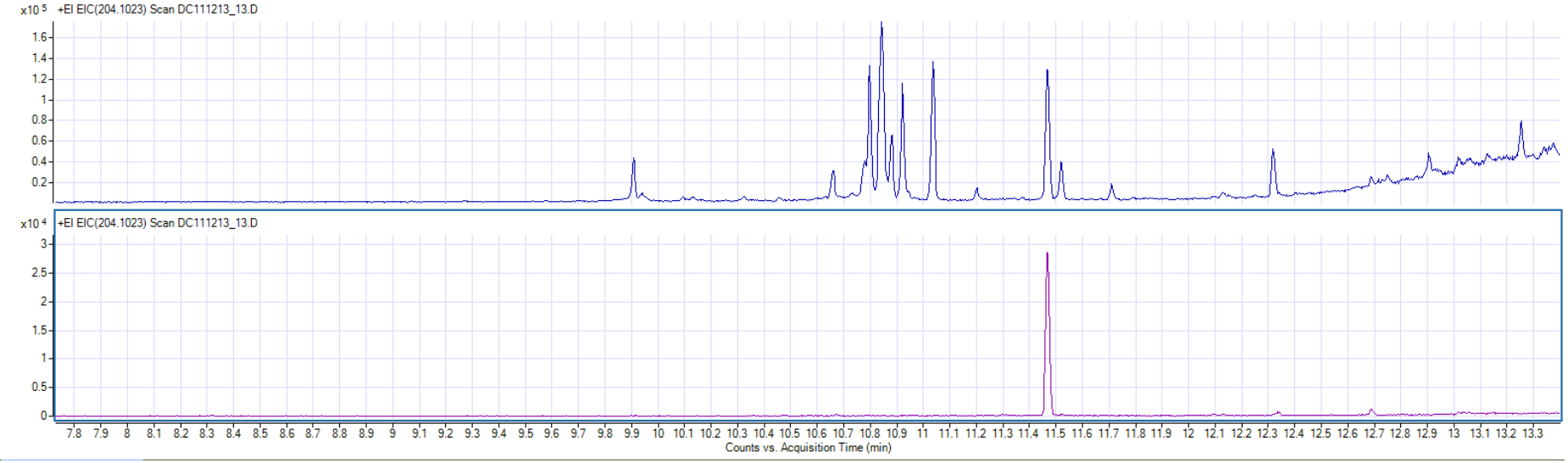
Pirimiphos-Methyl 1ng-g to 250 ng-g



Pesticide analysis: December extract – dirty matrix

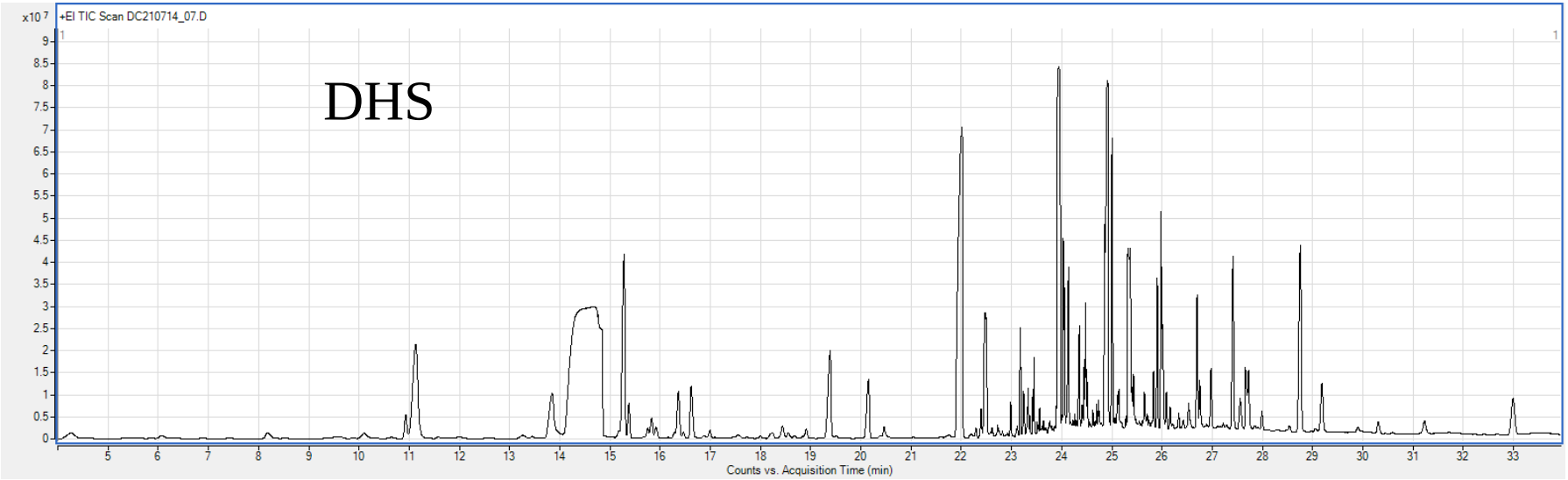


Clomazone comparison unit mass to 20ppm window



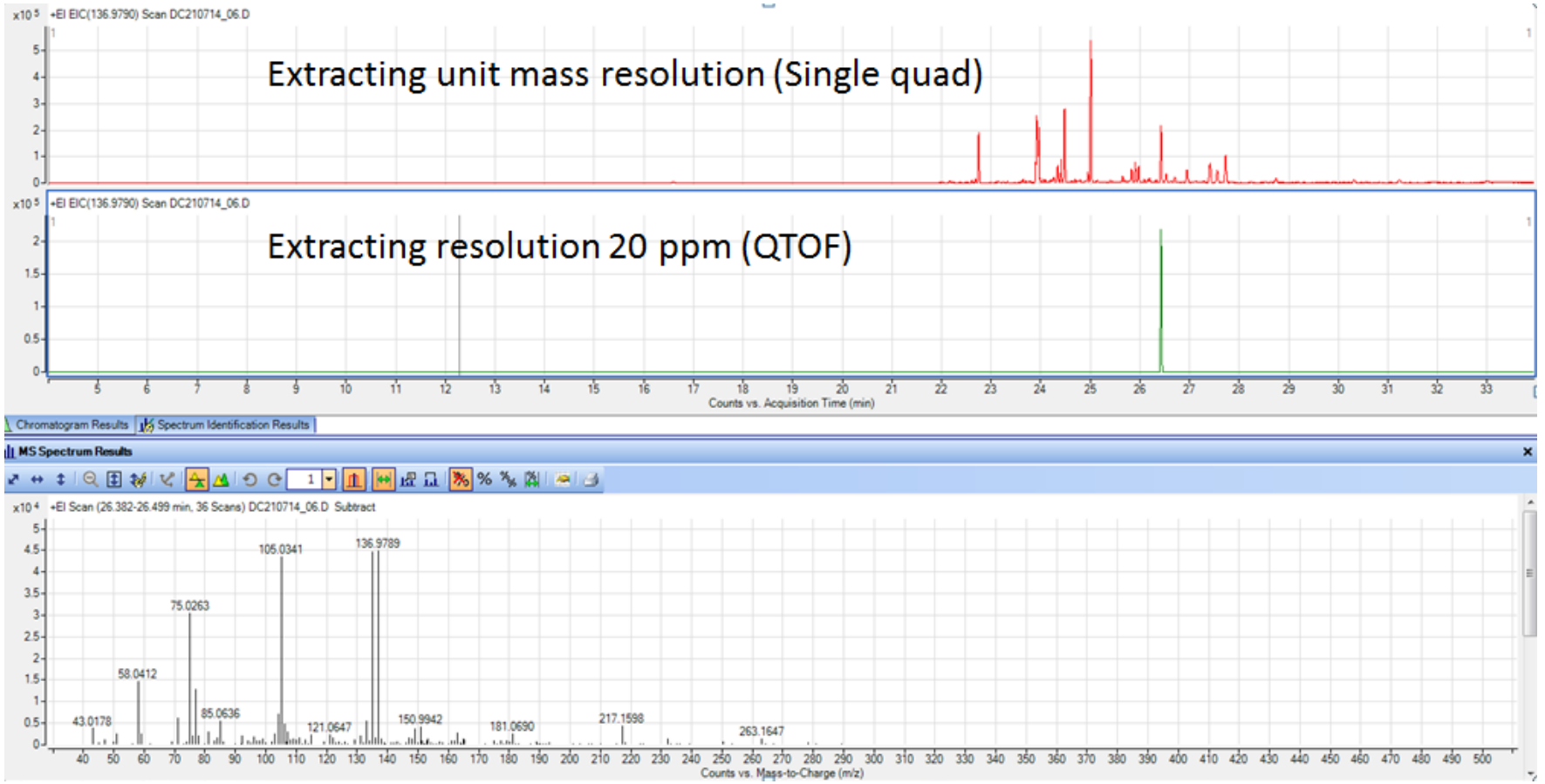
TOF vs Single Quad data

- Complex TIC chromatogram of whisky



- Look for a key analyte extract most abundant mass

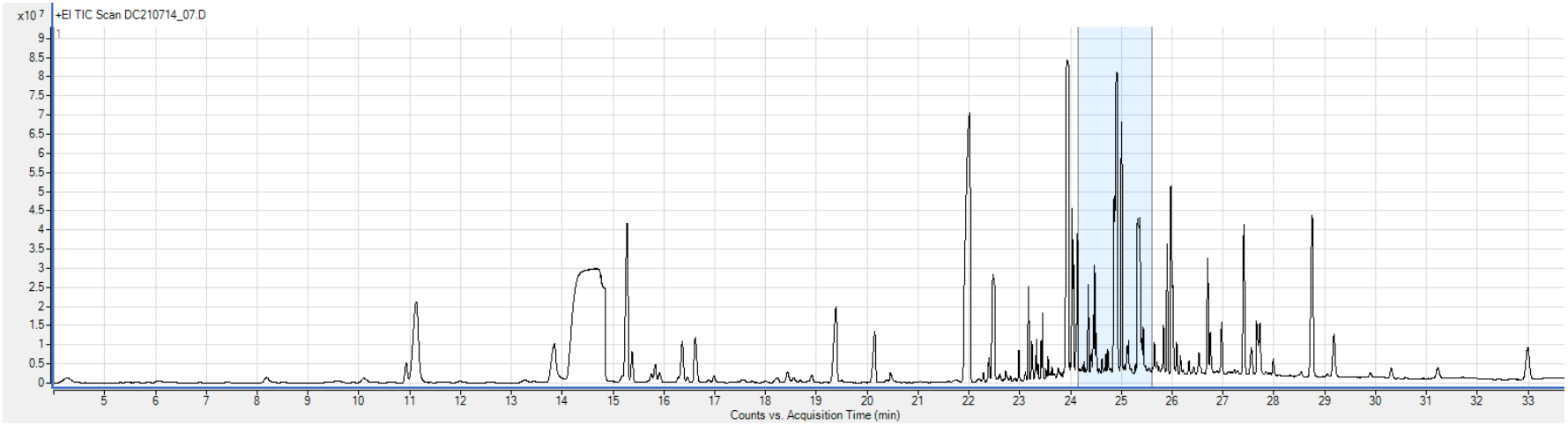
TOF vs Single Quad data



Improved selectivity and signal to noise

Deconvolution

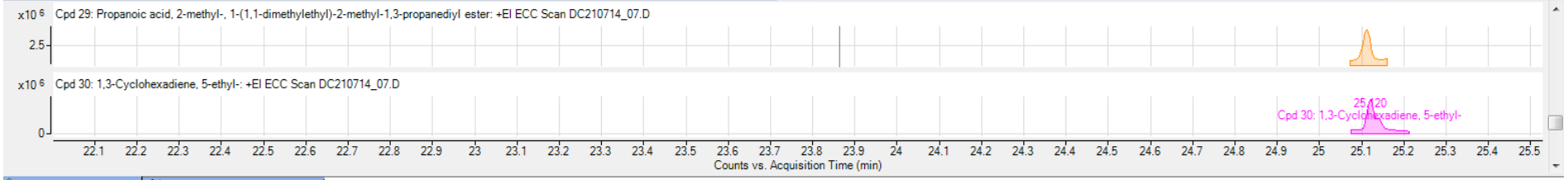
- DHS – Spirit



- More data points across a peak (5-10 hz) compared to 3hz single quad
- Deconvolute with sub-unit mass resolution

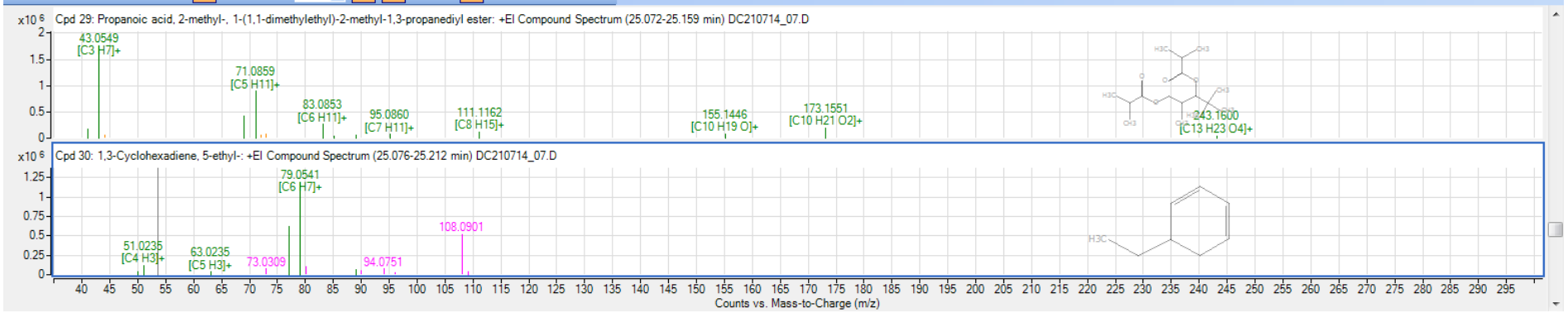
1,3-Cyclohexadiene, 5-ethyl-	☑	30	Cpd 30: 1,3-Cyclohexadiene,...	C8H12	64.47				108.094				79.0541	79.0541	Pos
Cyclopentaneethanol, .beta.,2,3-trimethyl-	☑	28	Cpd 28: Cyclopentaneethano...	C10H20O	75.12				156.151				69.0702	69.0702	Pos
Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-...	☑	29	Cpd 29: Propanoic acid, 2-m...	C16H30O4	69.43				286.214				43.0549	43.0549	Pos

Chromatogram Results



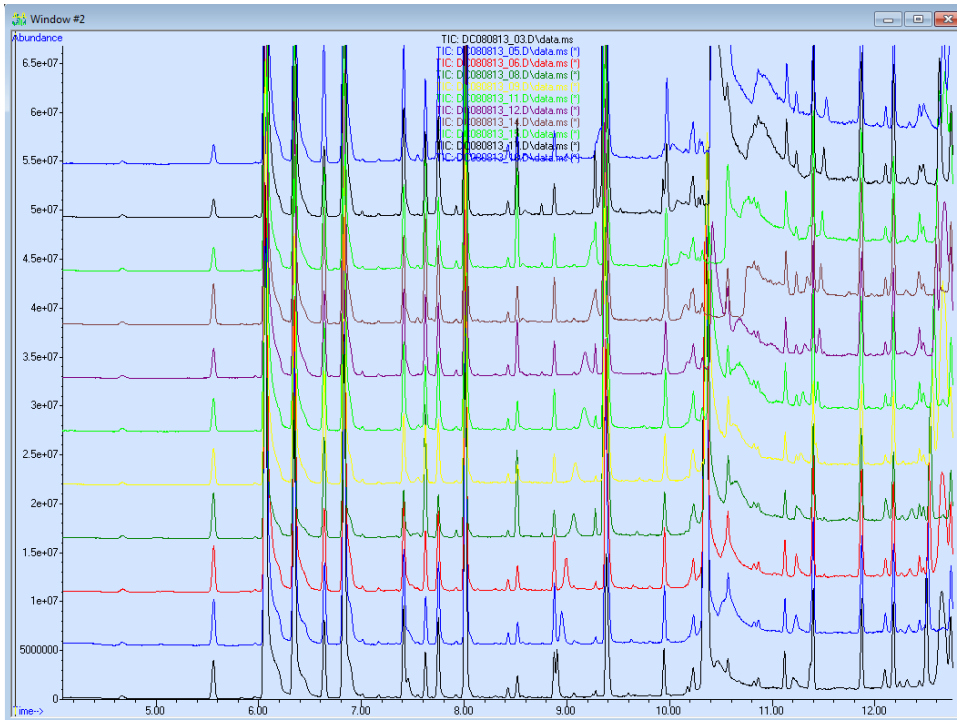
Chromatogram Results | Spectrum Identification Results

MS Spectrum Results

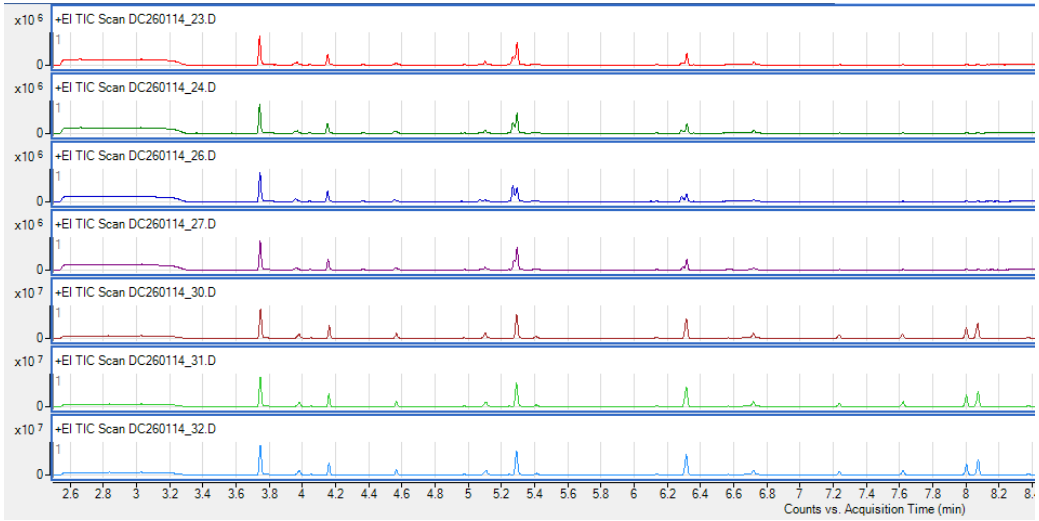


Mass Profiler Professional

- Finding differences between multiple chromatograms can be challenging
- PCA analysis key trends in data



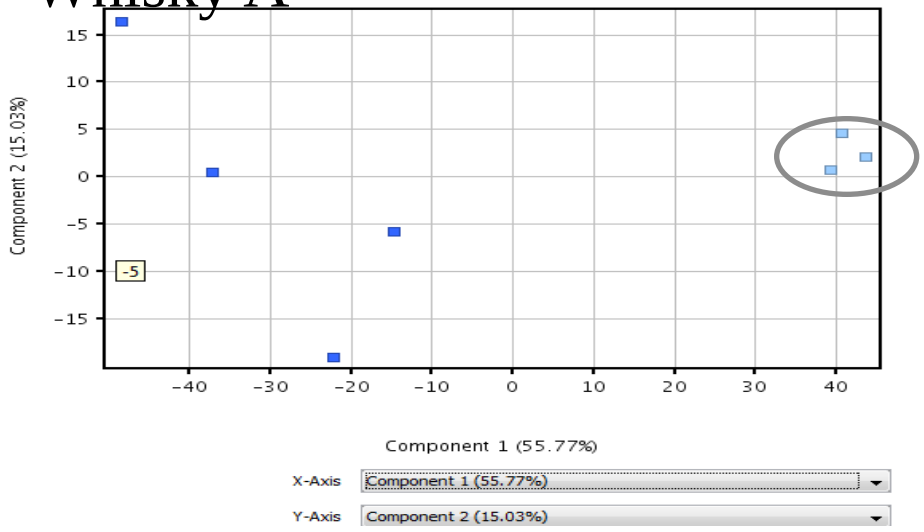
TIC – Whisky samples



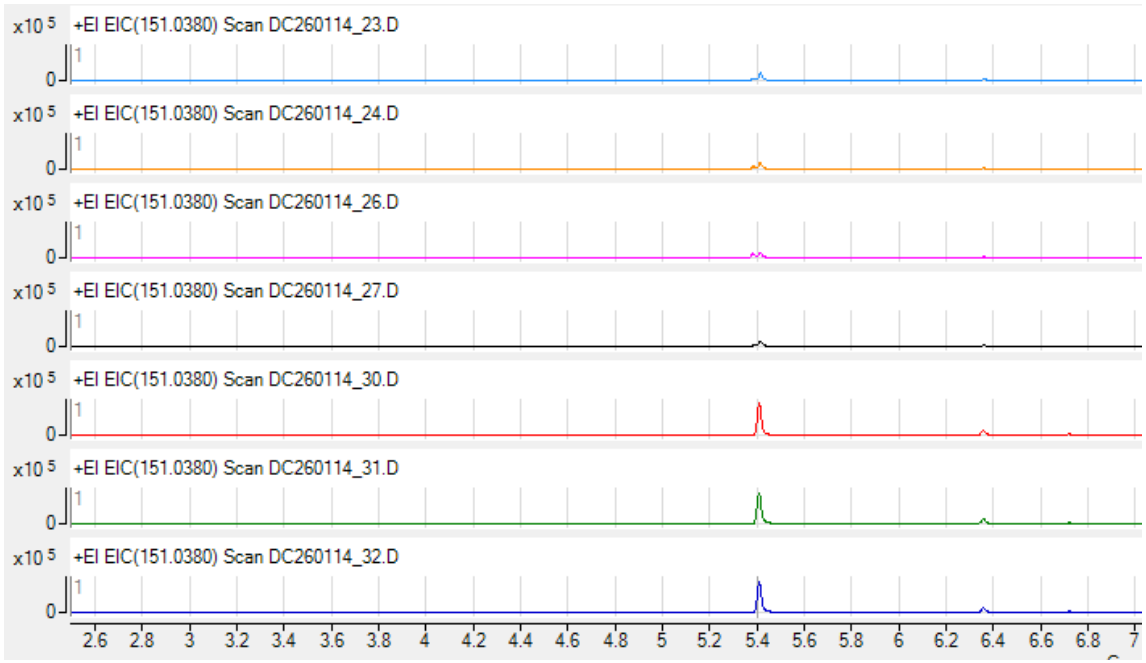
Whisky A

Whisky B

Whisky A

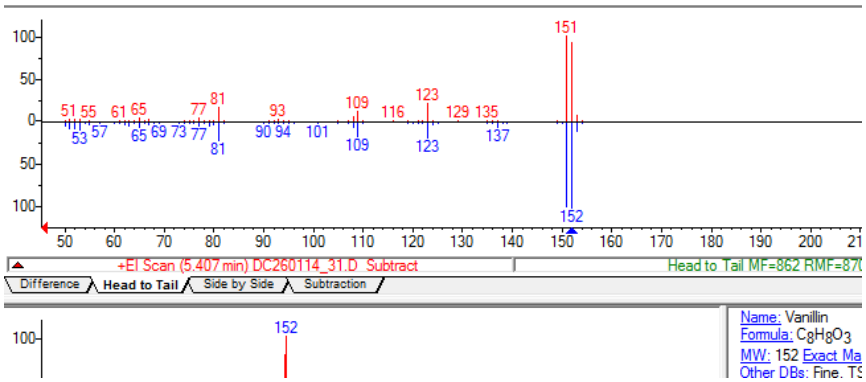


Whisky B

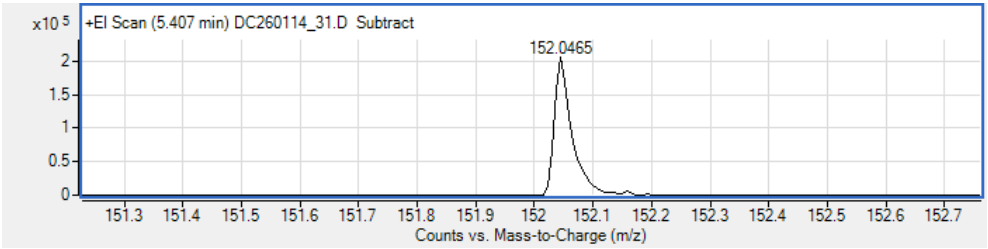


Whisky A

Whisky B

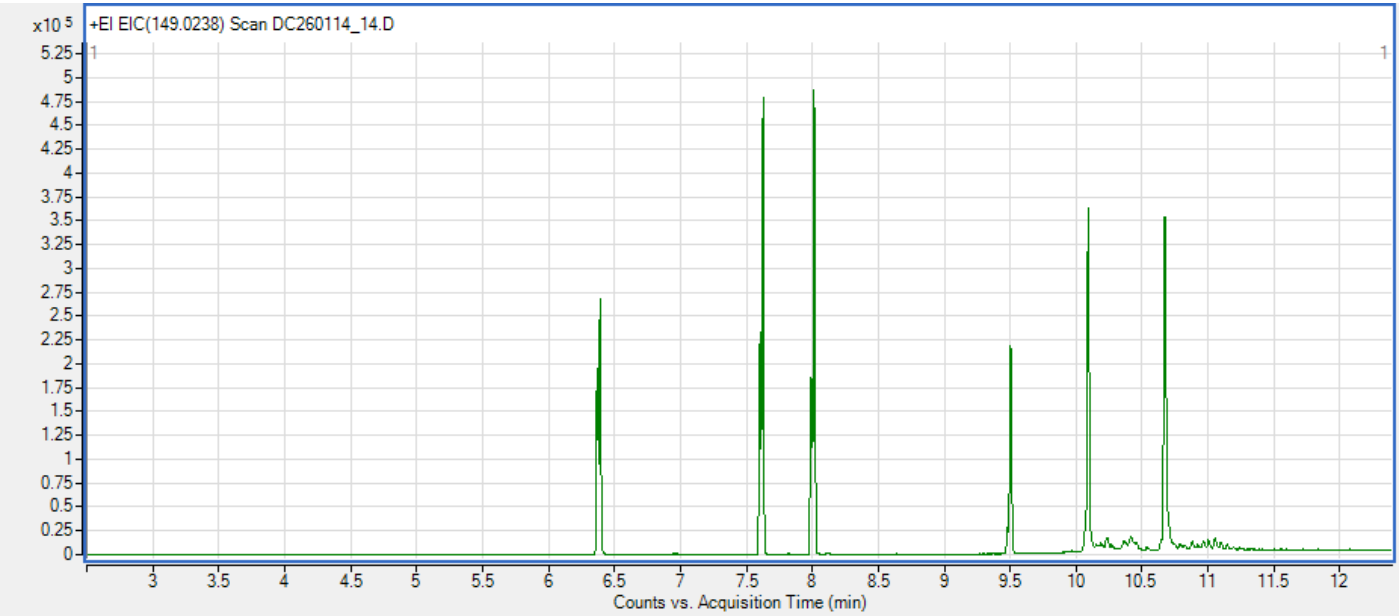


152.0465.....152.0473 5ppm



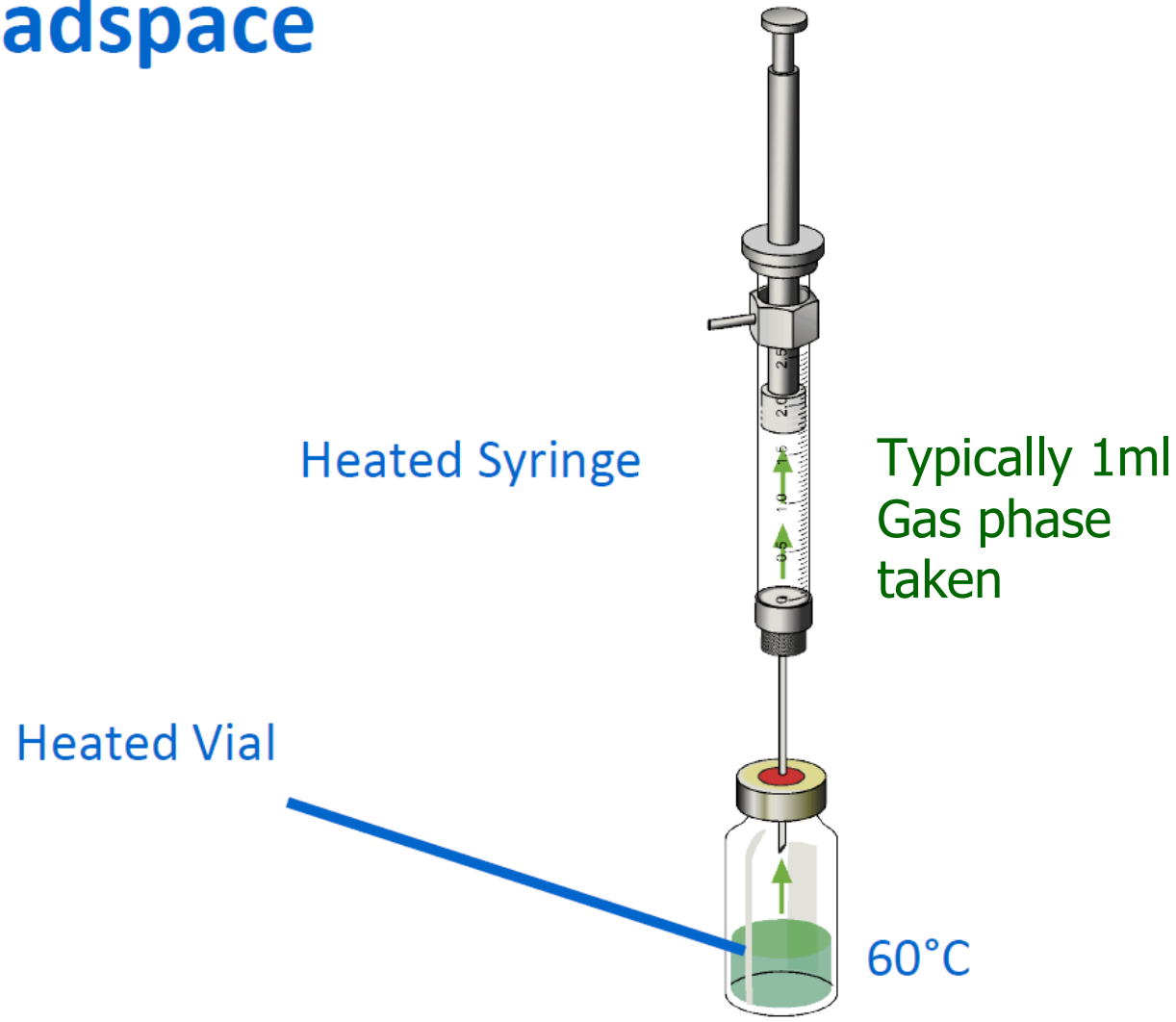
Whisky spiked with Phthalates at 1ppm

Extracting Characteristic 149 ion (with 20 ppm MS window)

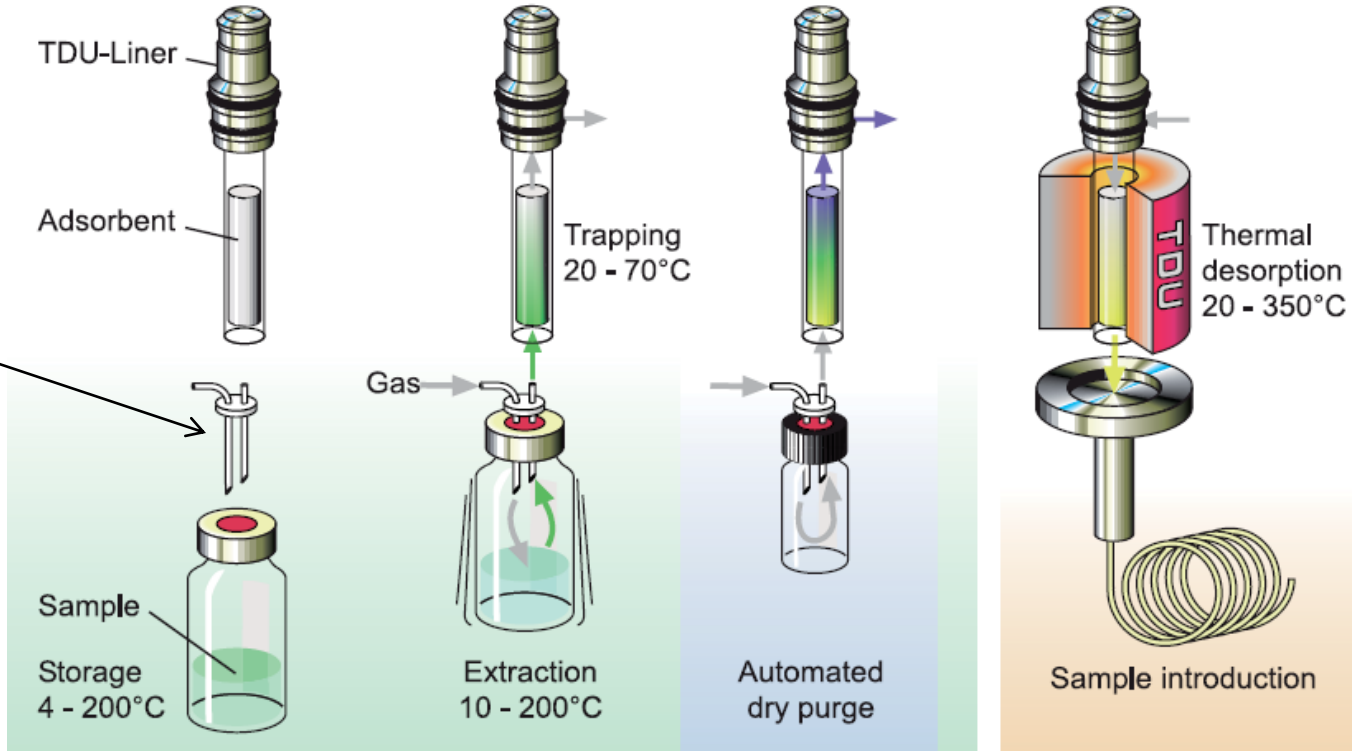


Dynamic Headspace

Static Headspace



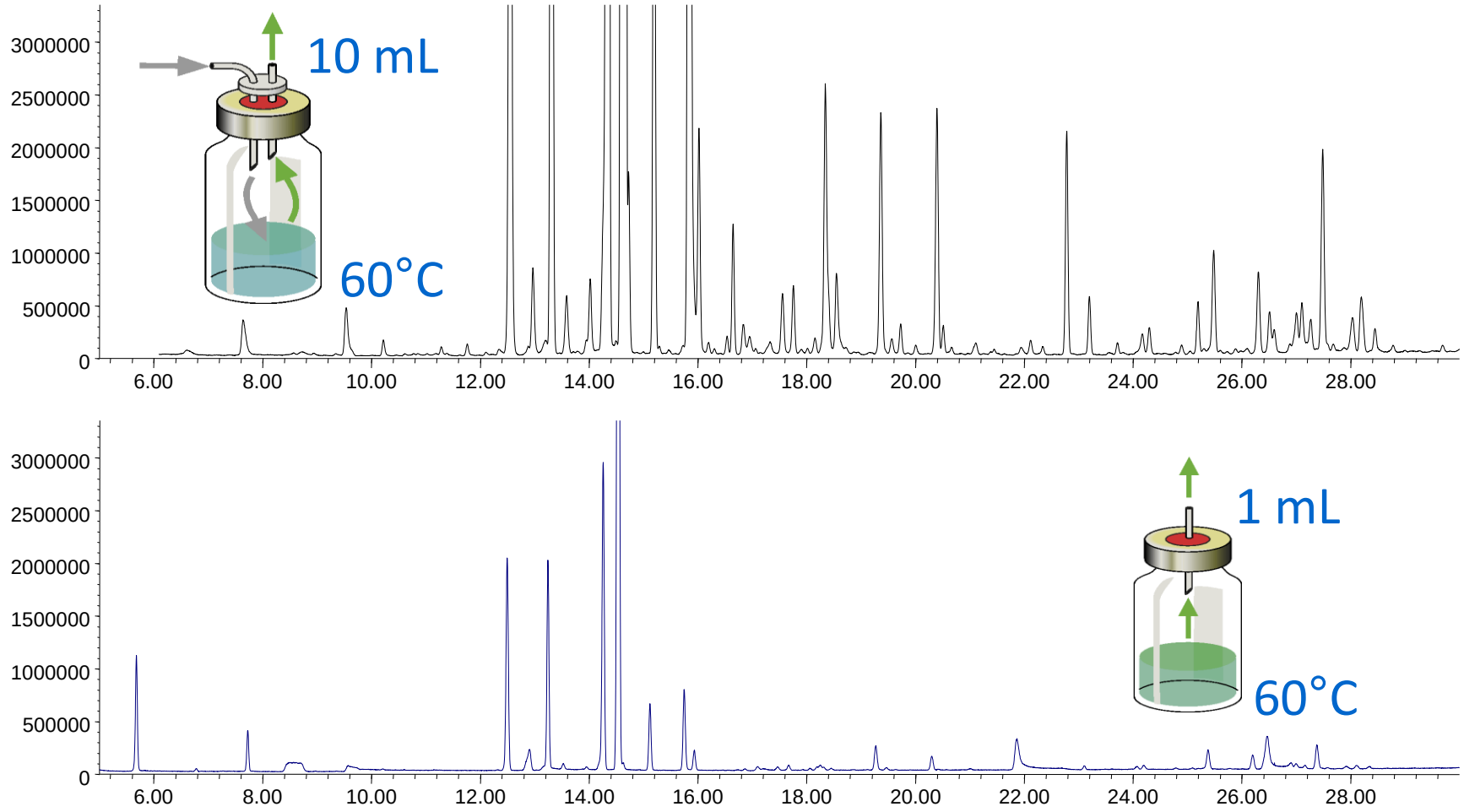
Double needle



Incubation and agitation

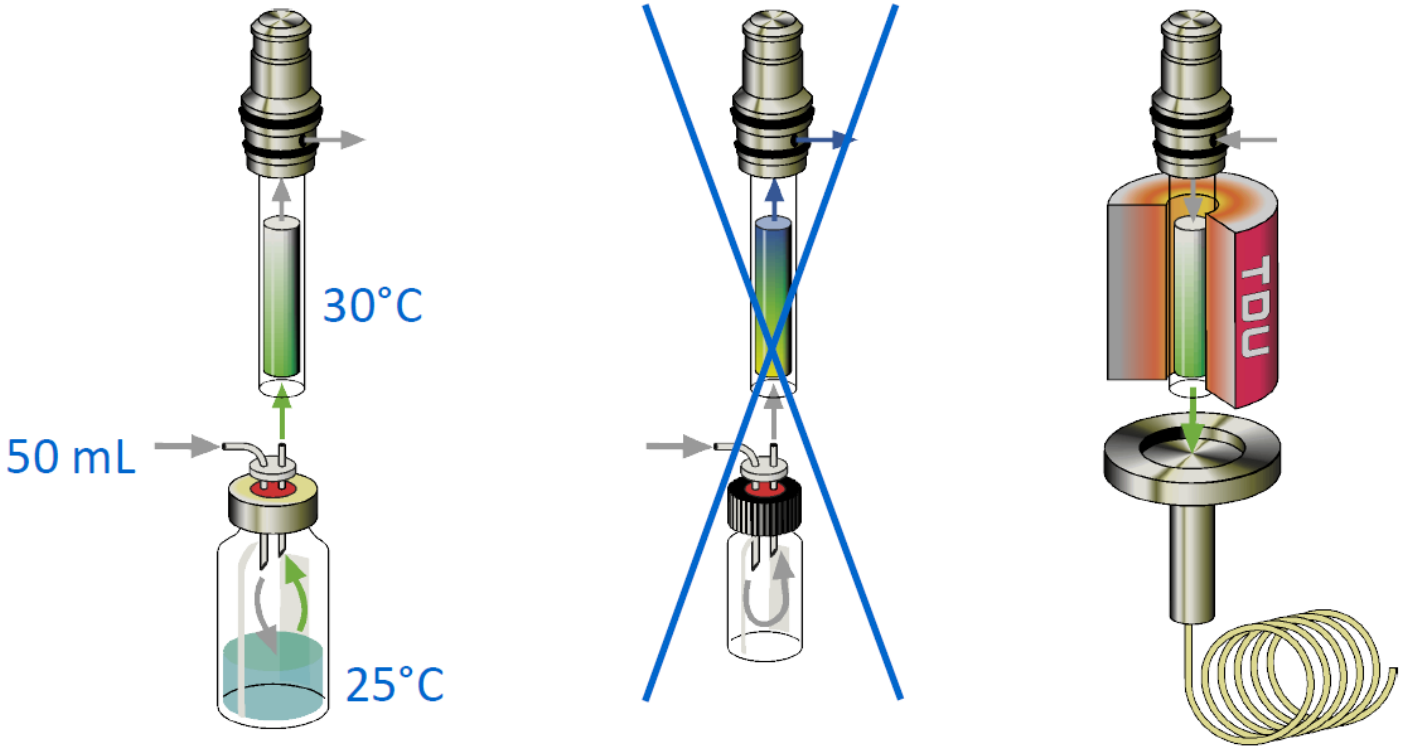
Trap moves down onto needles

Dynamic vs Static Headspace
Gin, split 1:10



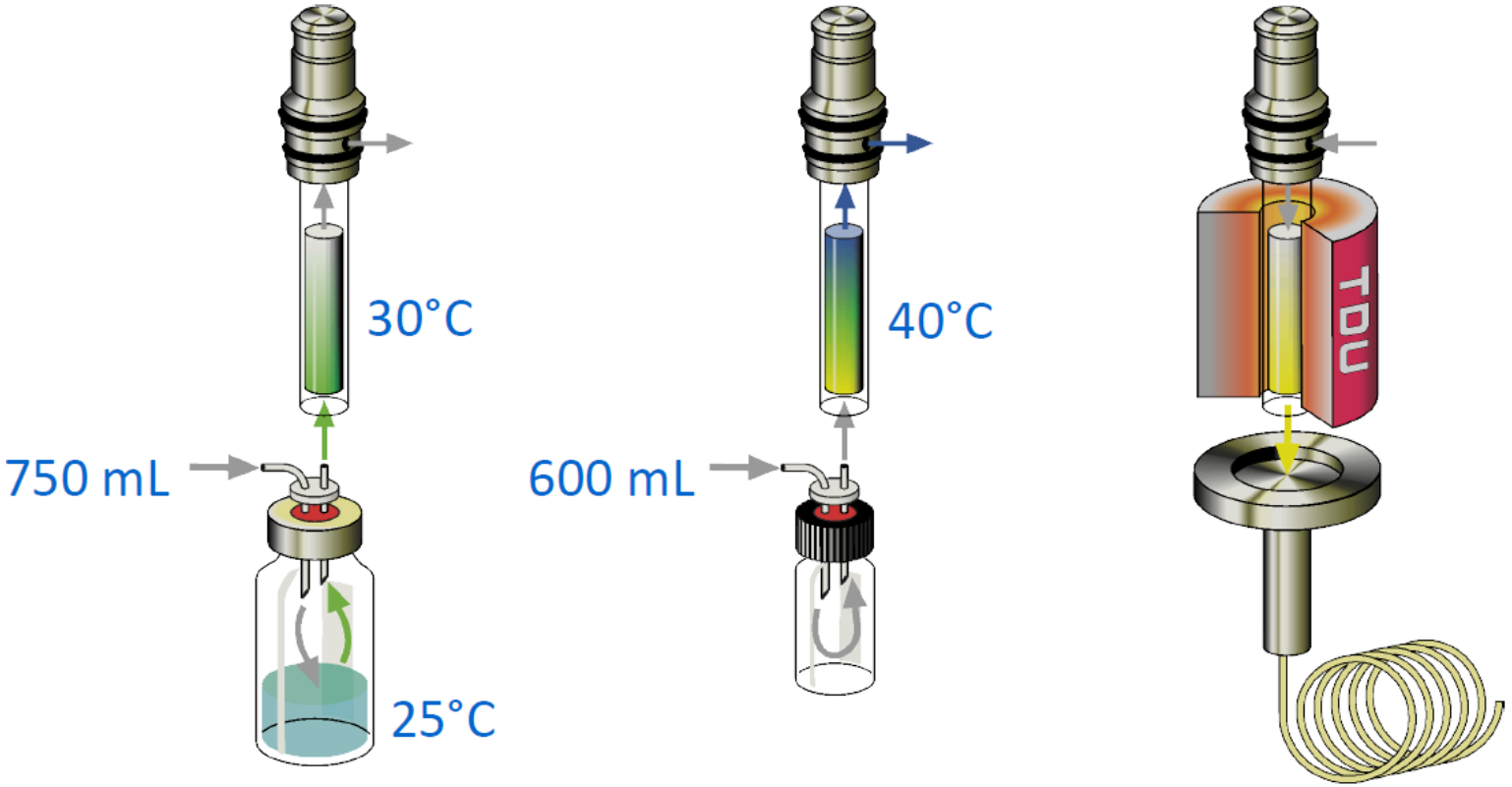
Dynamic Headspace

Method 1: Very Volatile Analytes



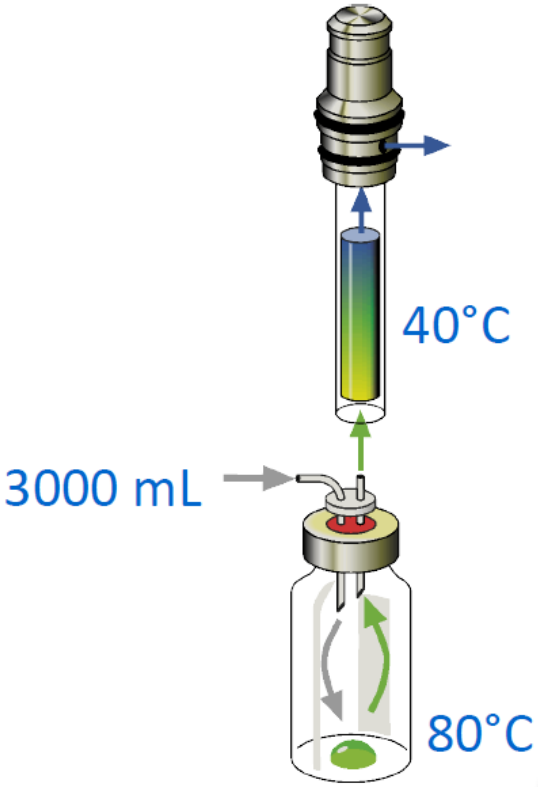
Dynamic Headspace

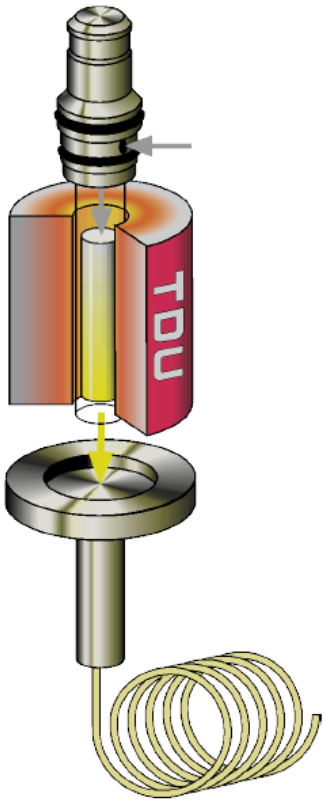
Method 2: Volatile or Semi Volatile Analytes



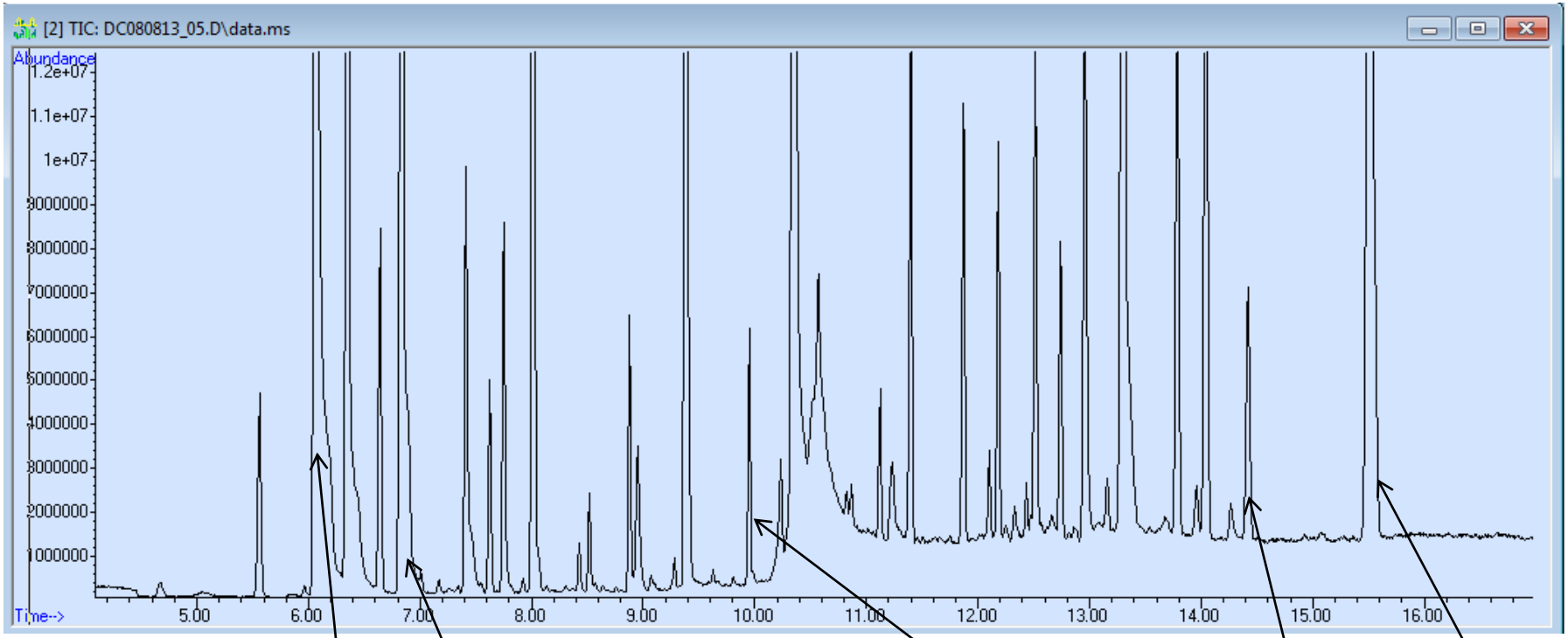
Dynamic Headspace

Method 3: Volatile, non volatile and hydrophillic analytes





- Frutarom – Flavour and ingredients company
- Working with Darren Caven-Quantrill (Flavour Chemistry Manager)
- Full Evaporative Technique (FET) on a number of flavoured drinks
- Current method – Manual extraction 2-3 hours



1-Butanol, 3-methyl-acetate

Butanoic acid ethyl ester

Linalool

4-Decalactone

Piperonal

Herbal based Liquor (35% alcohol)

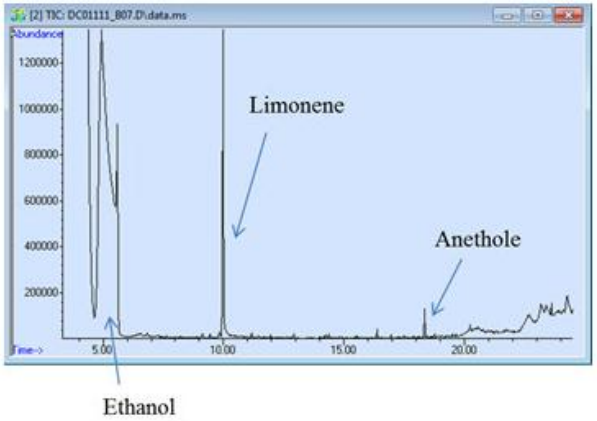


Figure 3(a) Herbal based liquor using Static Headspace

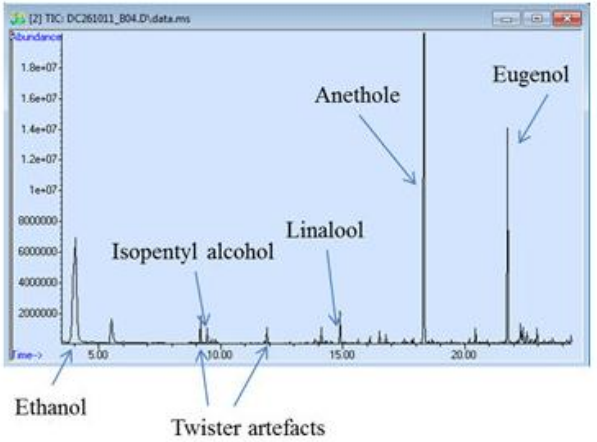


Figure 3(c) Herbal based liquor using Twister SBSE

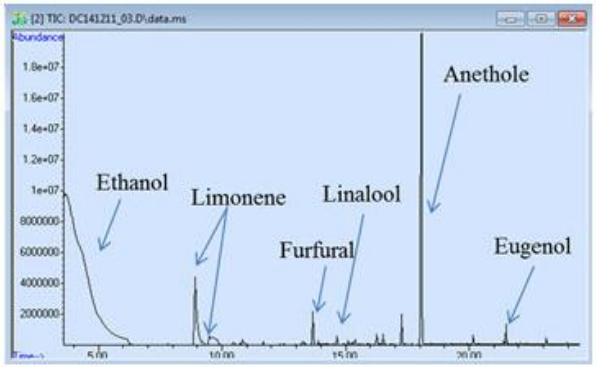


Figure 3 (b) Herbal based liquor using Headspace SPME Static Headspace

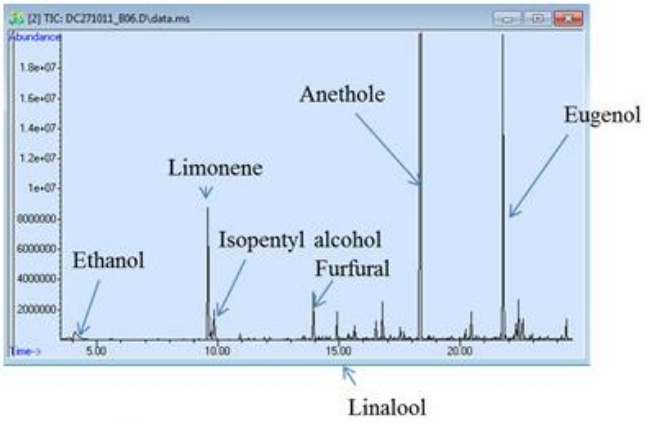
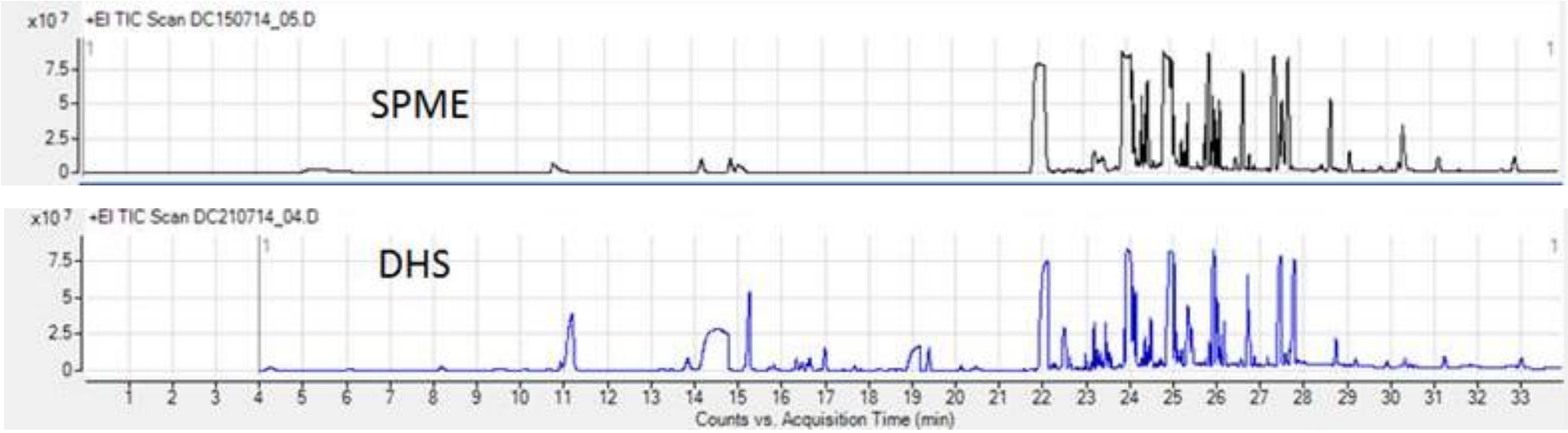


Figure 3 (d) Herbal based liquor using Dynamic Headspace

Method improvement – enriching analytes in distilled spirit



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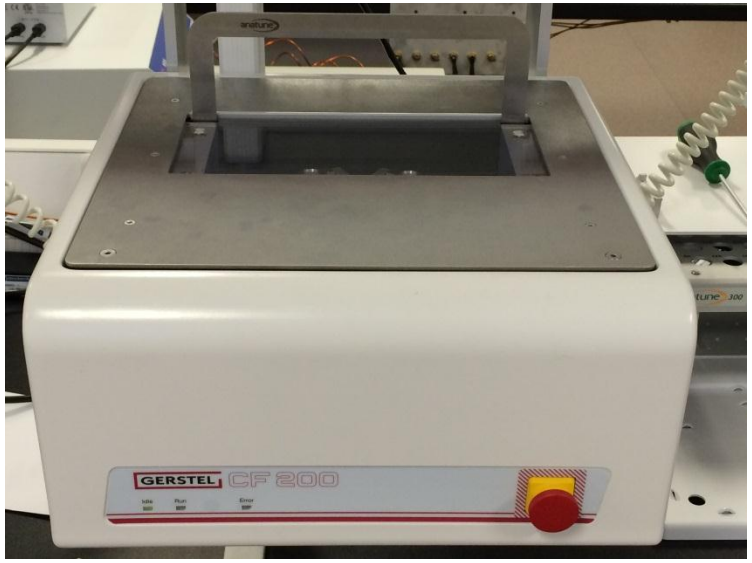
Briefly mention

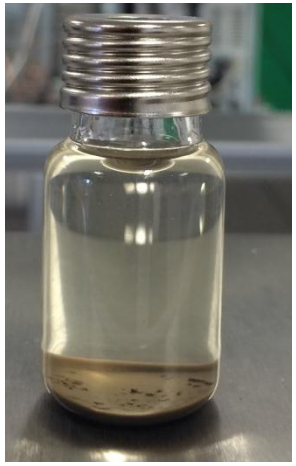
CF200

CF200 (Centrifuge and mixer)

Before centrifugation



After Centrifugation



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