





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

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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







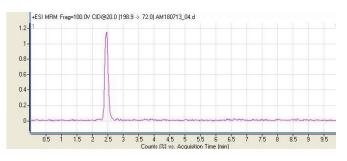




ITSP & LC/MS/MS

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

Chromatogram Monuron (quantifier transition) in standard 0.40 $\mu g/L$ after extraction



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









Summary of Presentation

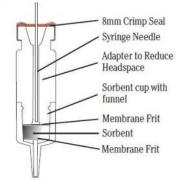
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Instrument Top Sample Preparation



- 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







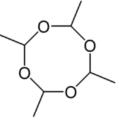


Harm - NDMA



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

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

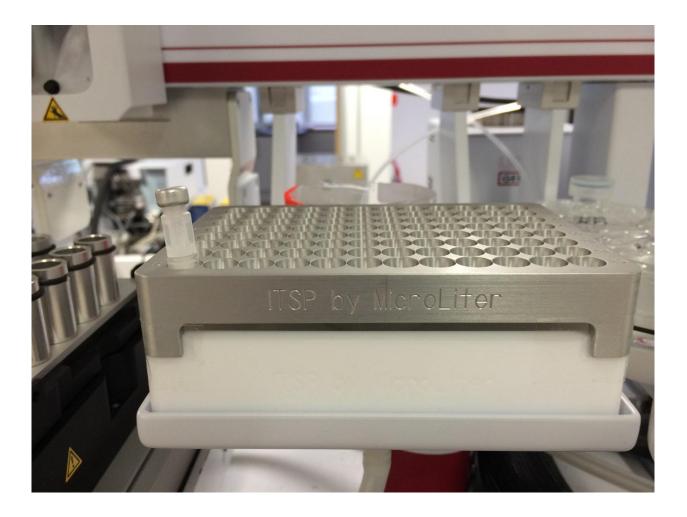








Close up of Tray









Automated Sample Prep10 ul Syringe2.5 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 μ l dichloromethane

1000 µl of methanol

Equilibrated 2000 μI of HPLC grade water

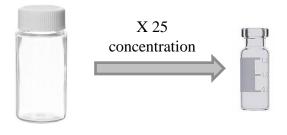
Load 10 ml of sample (in water)

Dried 15 minutes

Eluted 400 ul dichloromethane

Left MPS (10 ul) Large Volume injection





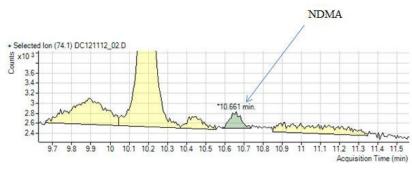






GC/QqQ

- GC/MS triple quad Application
 - Increased Sensitivity and Selectivity
 - Direct comparison at NDMA at 0.125 ng/ml (without extraction)



Single Ion Monitoring





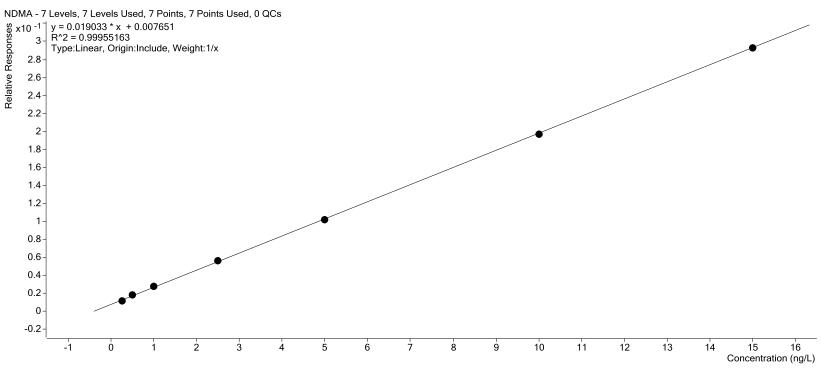
Multiple Reaction monitoring



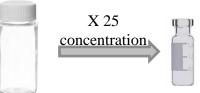




Extracted Water - NDMA



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

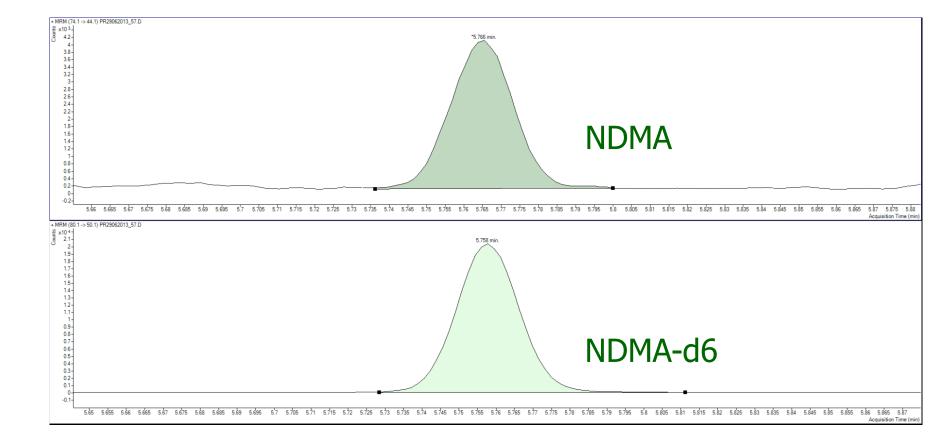








Standard Extracted Water 10 ng/l

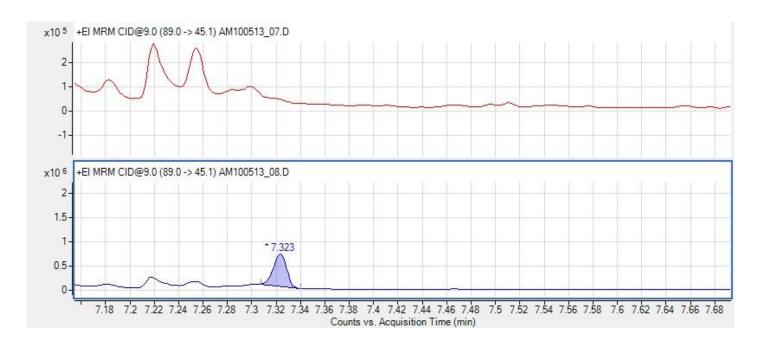








40 ng/l Metaldehyde extracted standard comparison with blank



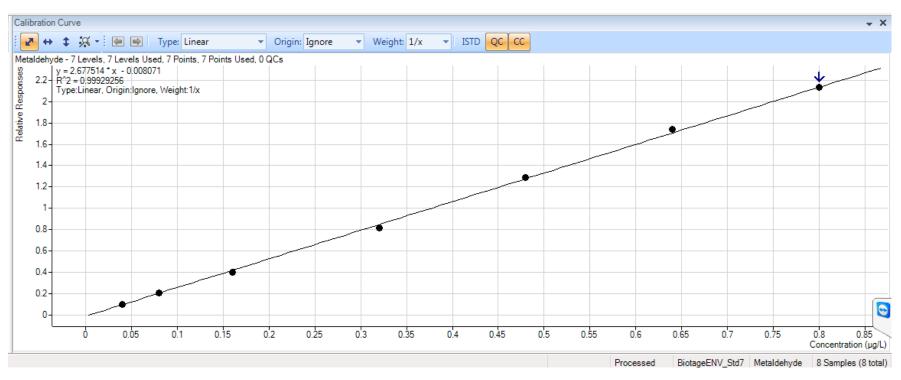
Detection limit approx 2 ng/l (based on signal to noise from this standard)







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 (μ g/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

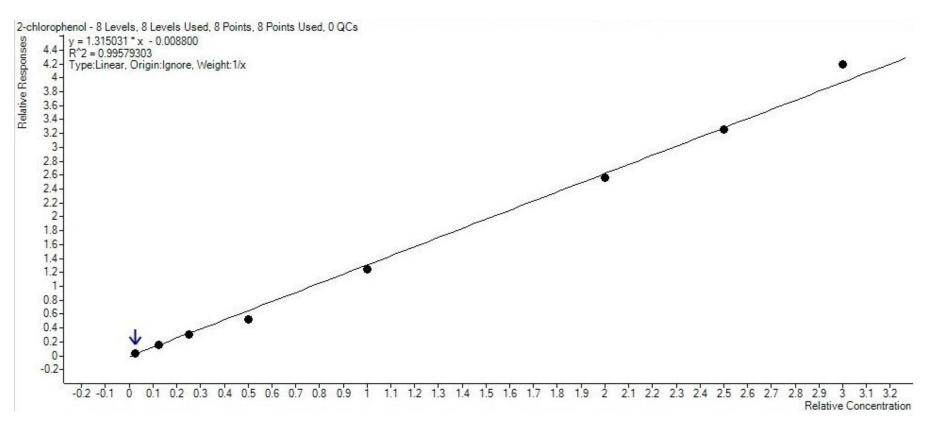






Severn Trent Water (STW) Bridgend -Collaboration

- 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

R²= 0.995 for 2-chlorophenol R²= 0.995 for 2-methylphenol R²= 0.992 for 2,4-dichlorophenol R²= 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
\$D	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



JUINCIR

V CHIMOI



Agilent Technologies

GERSTEL

Phases currently available

Stock Number Vendor Part # Bedmass Sorbent Substrate End-Capping Separation Modee 07-8.8AN10-20A Biotage 9603-0010 10 EVOLUTE ARN Polymeric Mixed (RP+SCX) 07-8.8AN10-20A Biotage 9203-0010 10 EVOLUTE ARN Polymeric Mixed (RP+SAX) 07-8.8AN0-20A Biotage 230-0025 10 ISULTE C8 Silica Endcapped Reverse 07-C1820-20A Orochem OCC18 20 Orpheus C18 Silica Endcapped Reverse 07-C1820-20A Orochem OCC18 30 Orpheus C18 Silica Endcapped Reverse 07-C1830-20A Orochem OCC8 10 Orpheus C8 Silica Endcapped Reverse 07-C8A10-20A Orochem OCCN 10 Orpheus C8A Silica Endcaped Reverse 07-JNE0A0-20A Jordi 40092 10 Gel DVB Polymeric WAX 07-JNE0A0-20A Jordi 40052		JOINCHIC	• Chidor						
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07-UBCXP10-20AUCTSSBCX10Styre Screen BCXPolymericSCX07-UC1810-20AUCTCEC1810Selectrasorb C18SilicaEndcappedReverse07-UC18U10-20AUCTCUC1810Selectrasorb C18SilicaReverse	07-UAQAX10-20A	UCT	CAQAX1	10	Selectrasorb QA w/ACI AX	Silica		SAX	QA + Acetate Counter Ion
07-UC1810-20A UCT CEC18 10 Selectrasorb C18 Silica Endcapped Reverse 07-UC18U10-20A UCT CUC18 10 Selectrasorb C18 Silica Reverse	07-UAX10-20A	UCT	CUQAX2	10	Selectrasorb QA + C8	Silica		Mixed (RP+SAX)	C8 + QA
07-UC18U10-20A UCT CUC18 10 Selectrasorb C18 Silica Reverse	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-UC810-20A UCT CUC08 10 Selectrasorb C8 Silica Reverse	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	07-UCOCC10-20A	UCT	CHARCOAL21	10	Coconut Charcoal	Other		Special	Carbon







Summary of Presentation

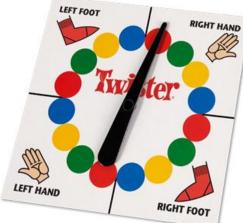
- Introduction
 - Anatune
- Automated (established) sample preparation techniques
 - ITSP (Instrument Top Sample Preparation)
 - Twister (SBSE)
 - ATEX (Automatic Tube Exchange)
 - DHS







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



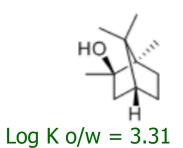






1. Theory of Twister SBSE

- Few examples (Methylisoborneol)



Twister Recovery Calculator			X		
File Tools Help					
	Sample Information				
	Sample Size (ml):	10 .			
Twister	CAS Number:	•	Q,		
Recovery Calculator	log K _{o/w}	3.31			
		C <u>a</u> lculate			
	Results				
	log K _{o/w}	3.31			
	Name	Unknown			
	Formula	Unknown			
wister	Twister		Recovery		
	10mm x 0.5mm		83.1%		
	10mm x 1.0mm		92.8%		
	20mm x 0.5mm		90.6%		
	20mm x 1.0mm		96.3%		
	7				

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

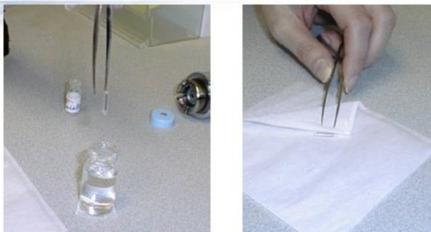








- Handling
 - After stirring for 2 hours
 - Remove with magnetic fish
 - Flush with few ml of deionised water
 - Wipe with a tissue
 - Insert into TDU Tube











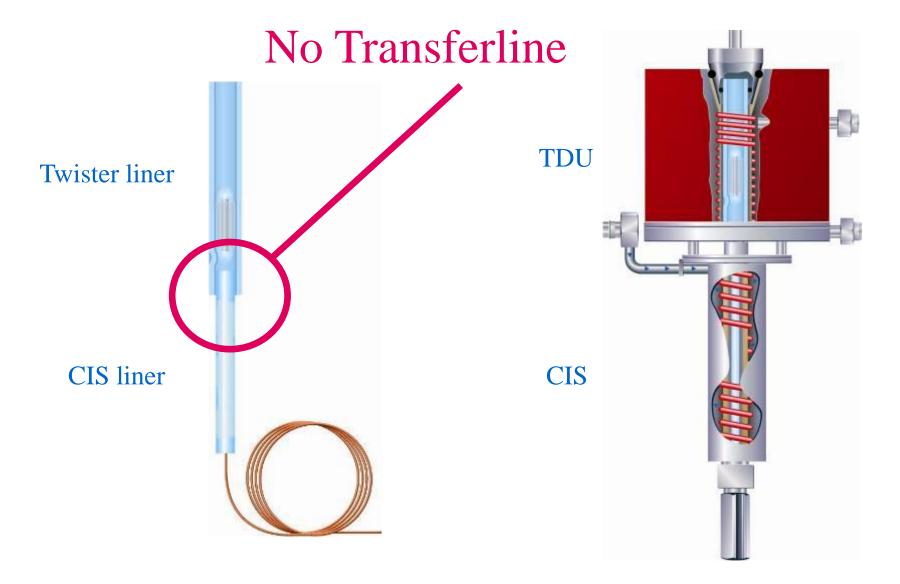
Twister Set up

















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.





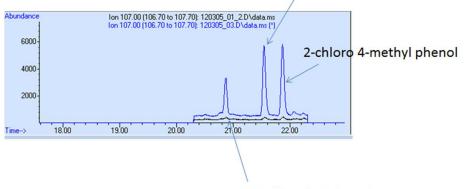


Twister Applications

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)

2-chloro 5-methyl phenol



2-6 dimethyl phenol



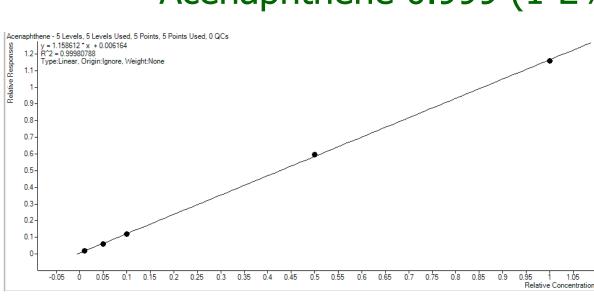




Twister SBSE – PAH solution

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

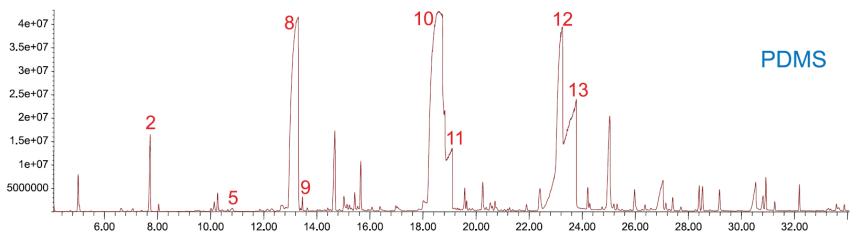


Figure 1. Whisky extraction chromatograms obtained using EG-Silicone, Acrylate and PDMS Twisters, non-polar column separation. 5 mL whisky sample (20 % EtOH (v/v), 1:1 dilution with water), 1000 rpm for 1 hour at room temperature. Peak identification: 1. Phenol; 2. C6 Acid ethyl ester; 3. o-Cresol; 4. 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.







Summary of Presentation

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



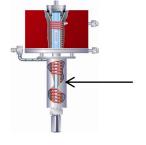




Look at volatiles in an involatile matrix

- 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)





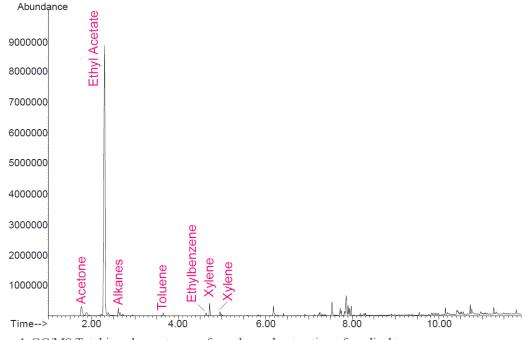
CIS liner kept clean







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)









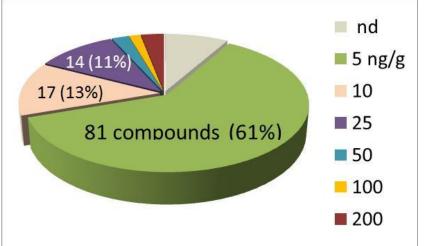


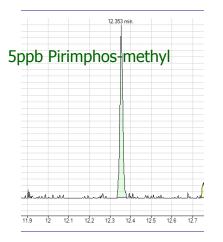
• Poster at EPRW





• 132 Spiked Pesticides

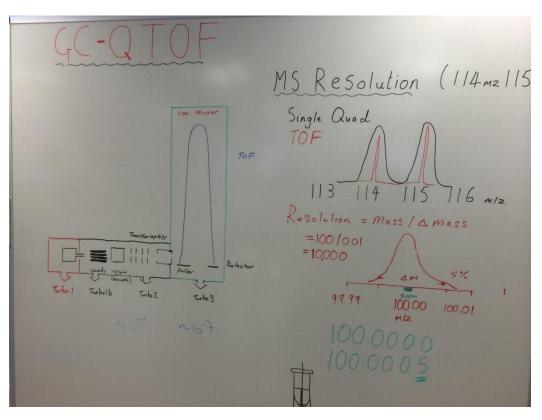










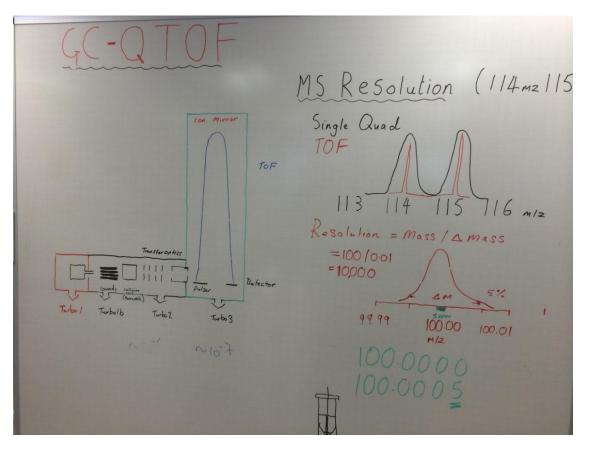


- Consistent Mass Accuracy= (Measured Mass-Theoretical)/ (Theoretical / 1000000) Based on mass 100 m/z
 - = (100.0005-100.000)/(100.000 / 1000000)
 - = 5 ppm
- Typical MS Resolution High 12000 Low 7000 (0.01 m/z wide)









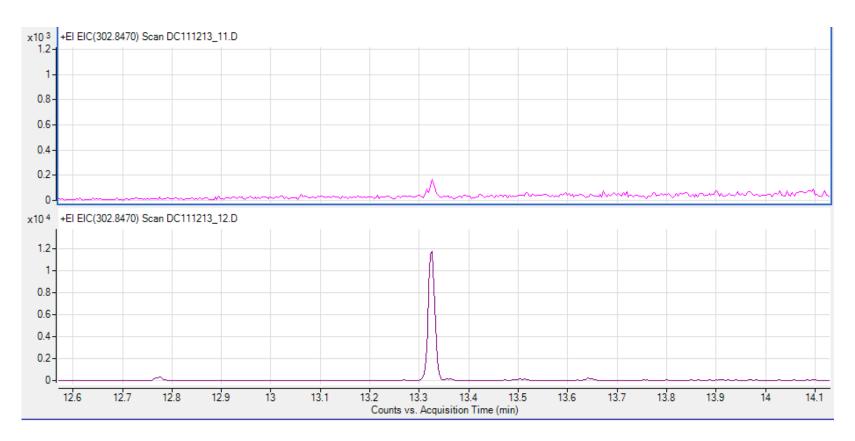
- Structure elucidation accurate mass
- Filter data with v low mass window to obtain good limits of detection
 - DL approaching MRM QQQ methods







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

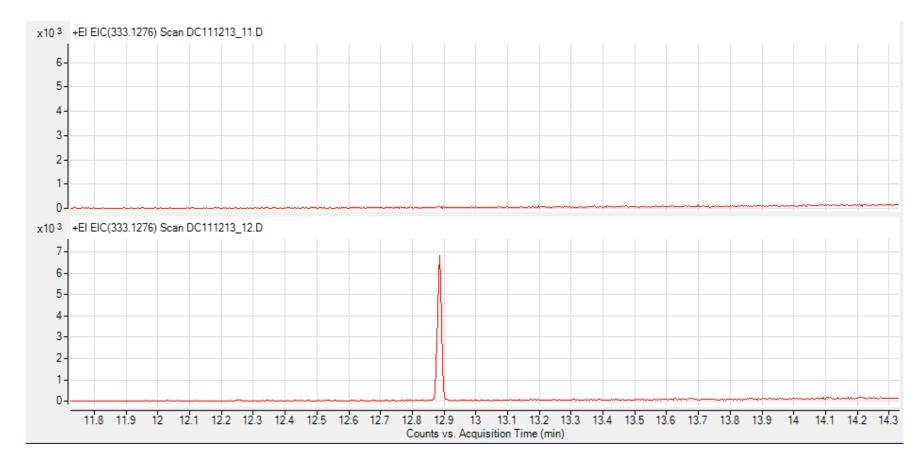








Extracted ion chromatogram of Primiphos-ethyl 10 ng/g in apple extract with 0 ng/g in apple extract

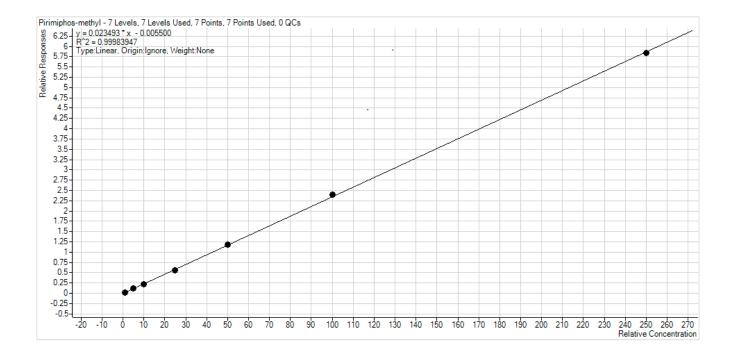








Pirimiphos-Methyl 1ng-g to 250 ng-g

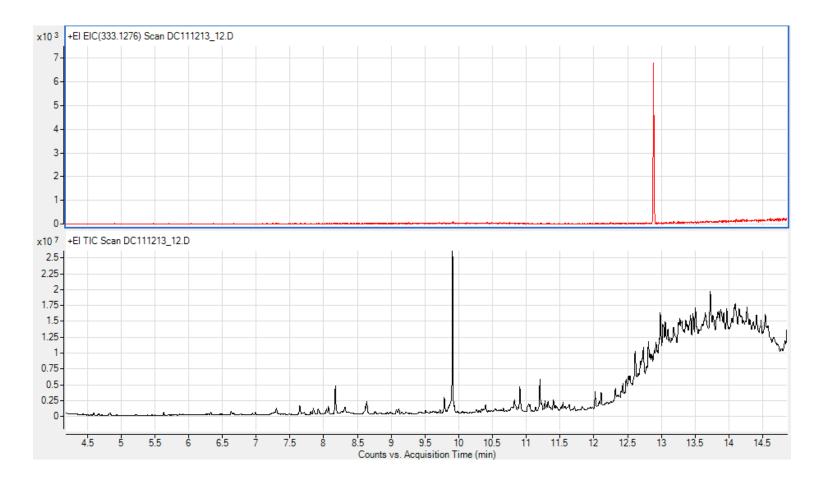








Pesticide analysis: December extract – dirty matrix

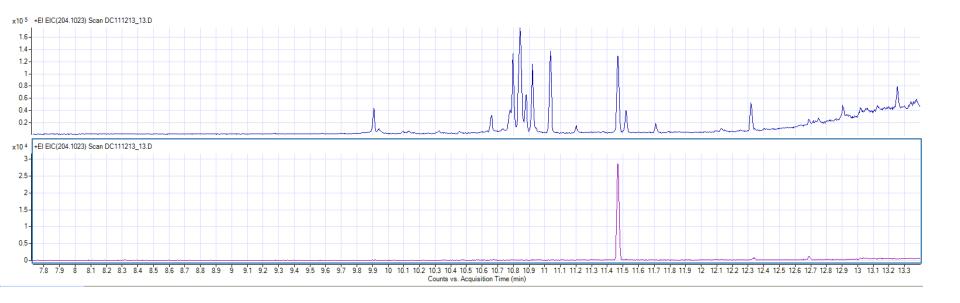








Clomazone comparison unit mass to 20ppm window

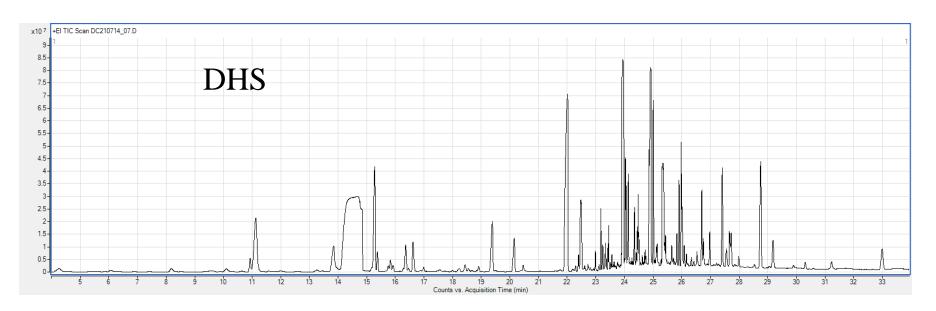








TOF vs Single Quad data Complex TIC chromatogram of whisky



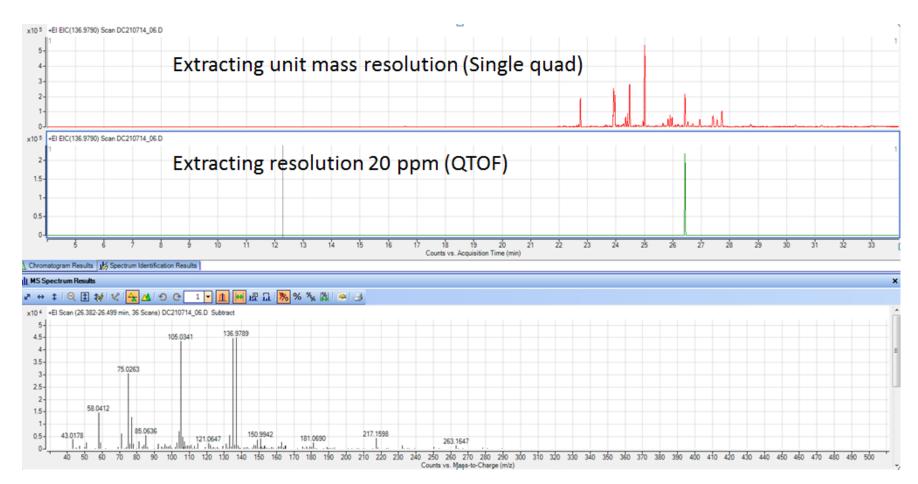
 Look for a key analyte extract most abundant mass







TOF vs Single Quad data



Improved selectivity and signal to noise

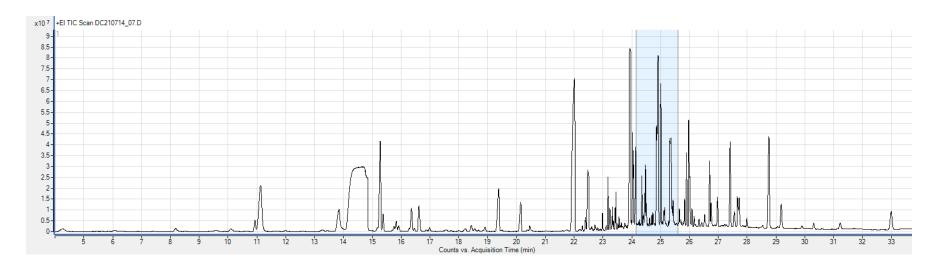






Deconvolution

• 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	•			Cyclohexadiene,	. C8H12	64.47			108.094								0541 Pos
Cyclopentaneethanol, .beta.,2,3-t	•			opentaneethano	C10H20O	75.12			156.15								0702 Pos
Propanoic acid, 2-methyl-, 1-(1,1-dimethyle	ethyl)-2 🔽	29	Cpd 29: Prop	panoic acid, 2-m	C16H30O4	69.43			286.214	•					43	3.0549 43	0549 Pos
<																	4
A Chromatogram Results																	
⊉ ↔ ‡ Q € ₩ 12 <mark>☆</mark> ▲ ₹	D C 2 🛛 🚧	🚺 🕰 🏄	Ł 🖉 📉 🧐	% 🗞 🕅 🛛	A Minutes	- I A	6										
x10 ⁶ Cpd 29: Propanoic acid, 2-methyl-, 1-(1,1	-dimethylethyl)-2-methy	yl-1,3-propane	adiyl ester: +El	ECC Scan DC21	10714_07.D												
2.5-															$\mathbf{\Lambda}$		
x10 6 Cpd 30: 1,3-Cyclohexadiene, 5-ethyl-: +E	ECC Scan DC210714	_07.D															
														Cpd 30: 1,3	25/120 -Cyclonexadien	e, 5-ethyl-	
22.1 22.2 22.3 22.4 2	2.5 22.6 22.7	22.8 22.9	9 2'3 2	23.1 23.2 2	23.3 23.4		8.7 23.8 23.9	24 24.1	24.2 24	3 24.4	24.5 2	4.6 24.7	24.8	24.9 25	25.1 25.2	2 25.3	25.4 25.5
						Counts vs.	Acquisition Time (m	in)									
▲ Chromatogram Results ▲ Spectrum Identification	tion Results																
MS Spectrum Results																	
🖉 🕂 ‡ 🔍 🗊 😻 🔽 🛧 🔼 🤄) ৫ - 👖	l 😝 😽 🛛	፲ 🔭 %	‰ 🔏 ⊨ 🚈 E	4												
x10 6 Cpd 29: Propanoic acid, 2-methyl-, 1-(1,	1-dimethylethyl)-2-meth	ıyl-1,3-propane	ediyl ester: +E	Compound Spe	 ctrum (25.072-2!	5.159 min) DC2107	14_07.D										
² 43.0549																	
1.5- [C3 H7]+ 71.0	950																
1- [C5H	11]+										0	0	.CH3				
0.5-	83.0853 [C6 H11]+ 9 [C	95.0860 27 H11]+	111.1162 [C8 H15]+			155.1446 [C10 H19 O]+	173.1551 [C10 H21 O2]+				нас	1013	3.1600 H23 O4]+				
	Compaund Spactrum	(25.076-25.21)	2 min) DC210	714 07 D								1 1				1 1	
v10.6 Cpd 30: 1.3-Cyclohexadiene 5-ethyl-: +F			2 11111 0 02 101														1 1 1
x10 ⁶ Cpd 30: 1,3-Cyclohexadiene, 5-ethyl-: +E 1.25-	79.0541																
	79.0541																
1.25 1 0.75 0.5 51 0225	79.0541	1	108.0901								нзс						
1.25- 1- 0.75- 0.5- 51,0235 63,0235	79.0541 [C6 H7]+	4.0751	108.0901								H3C						
1.25- 1- 0.75- 0.5- 51.0235 cn cm cm	79.0541 [C6 H7]+	4.0751	108.0901	120 125 130	135 140 145	5 150 155 160	165 170 175 18) 185 190 19	35 200 205 2	10 215 22		0 235 240	245 250	255 260 2	65 270 275	280 285	290 295

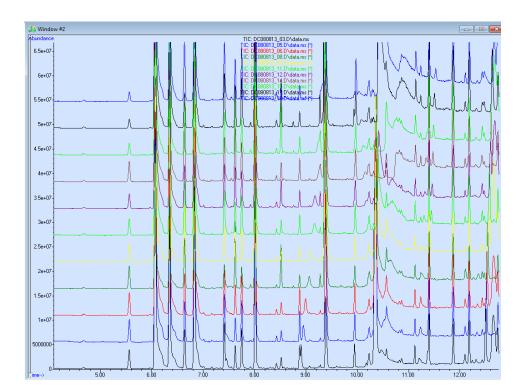






Mass Profiler Professional

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

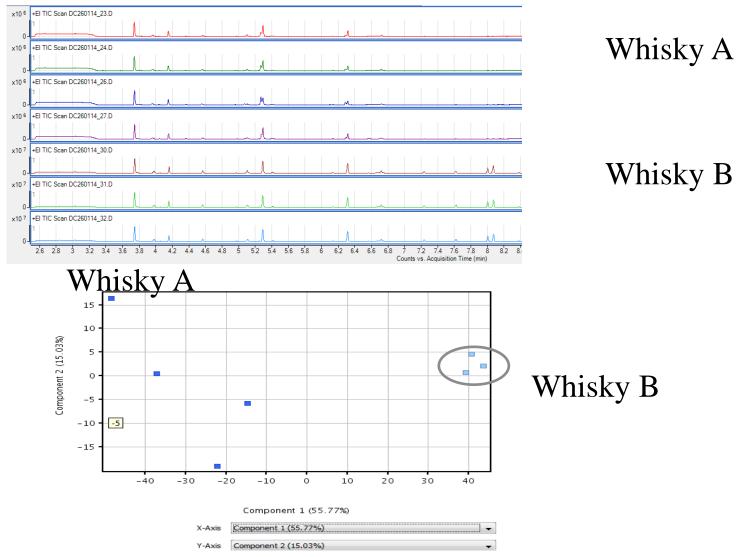








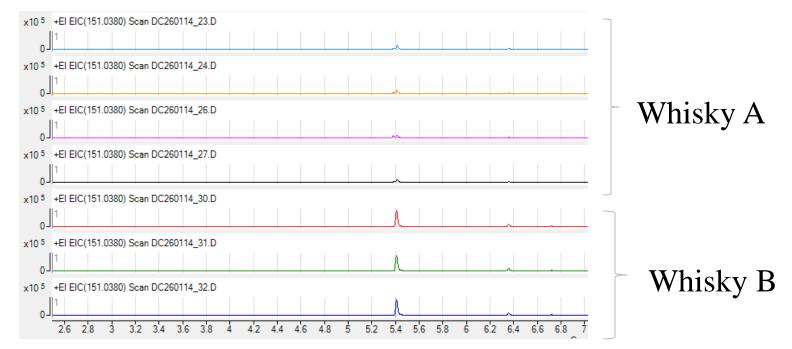
TIC – Whisky samples



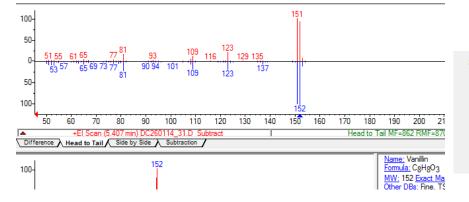


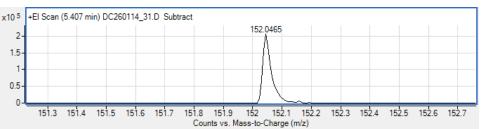






152.0465.....152.0473 5ppm





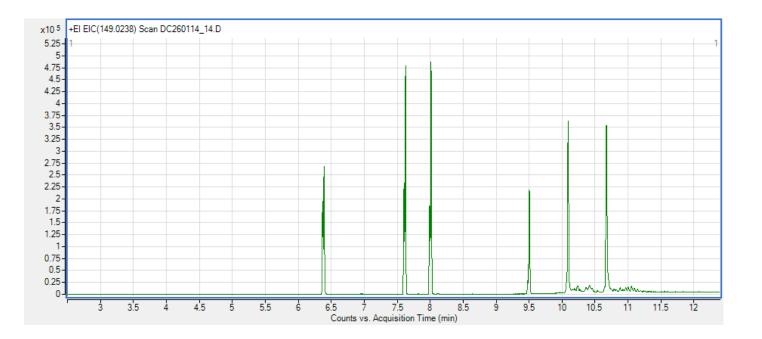






Whisky spiked with Phthalates at 1ppm

Extracting Characteristic 149 ion (with 20 ppm MS window)









Dynamic Headspace

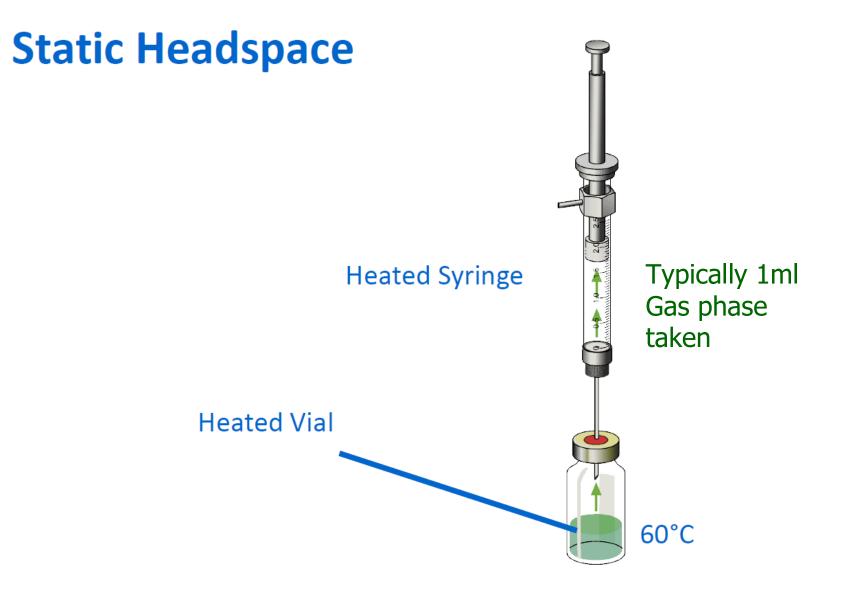








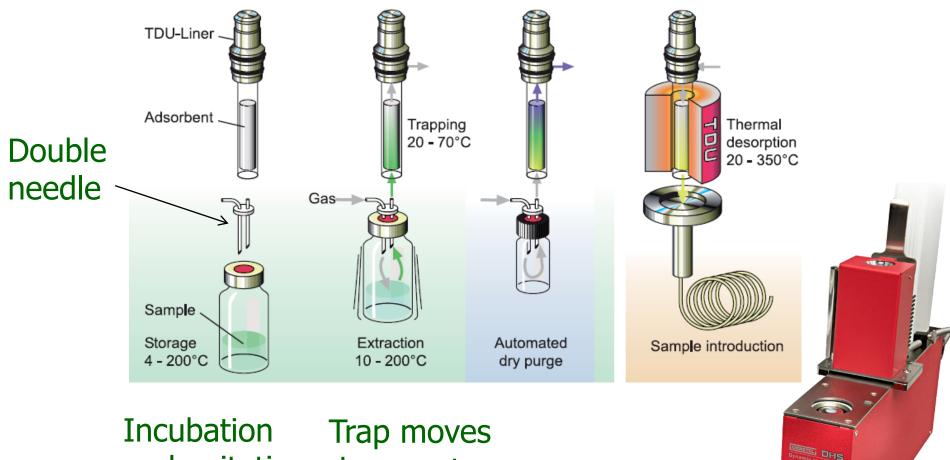




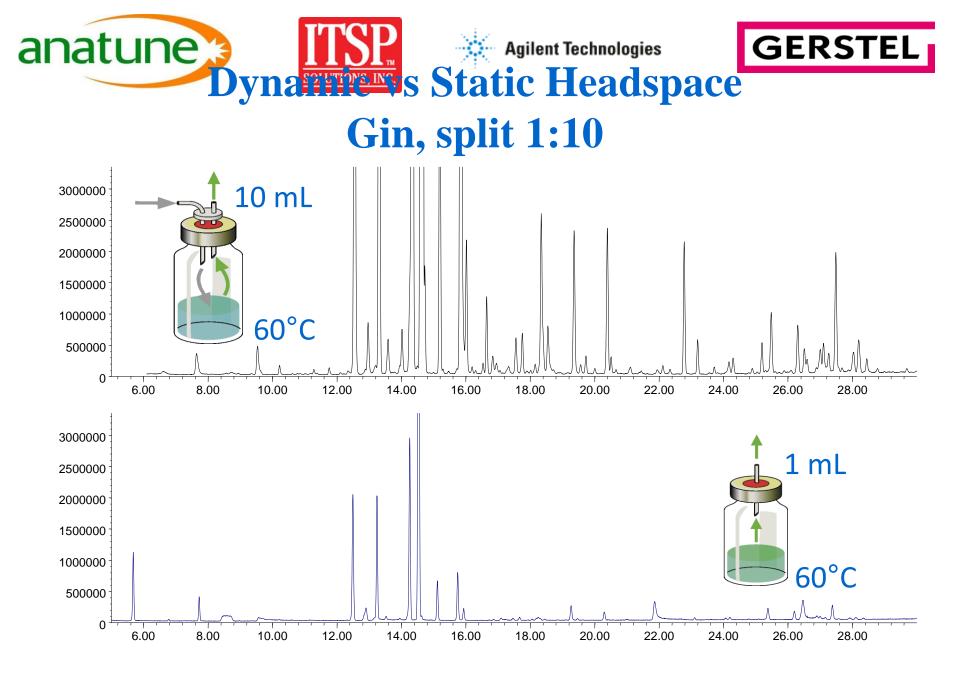








Incubation Trap moves and agitation down onto needles



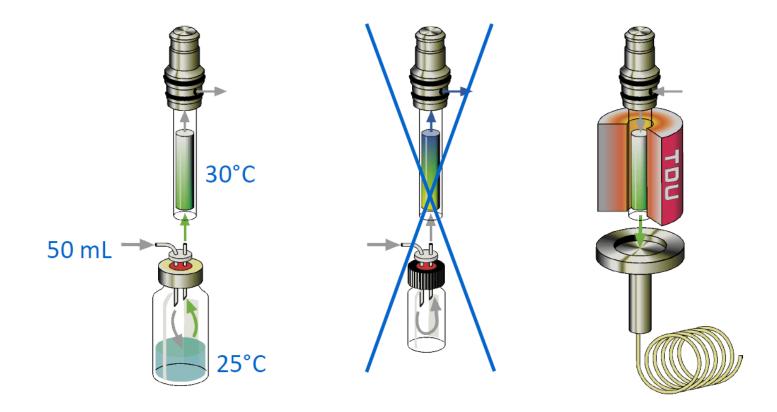






Dynamic Headspace

Method 1: Very Volatile Analytes



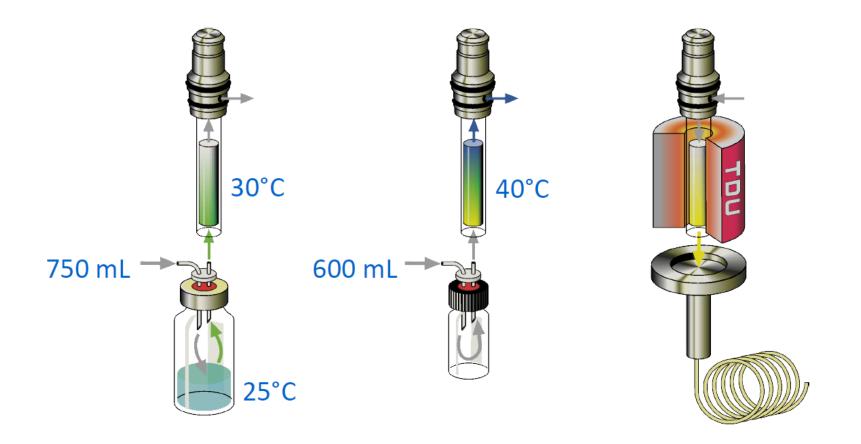






Dynamic Headspace

Method 2: Volatile or Semi Volatile Analytes



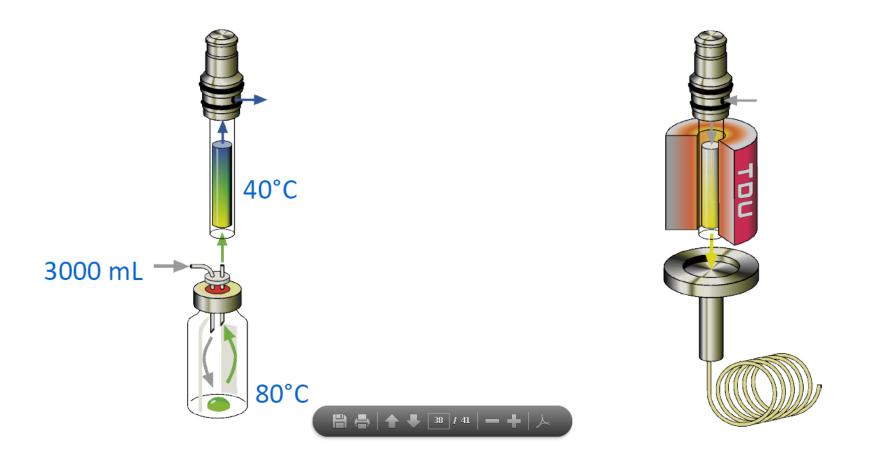






Dynamic Headspace

Method 3: Volatile, non volatile and hydrophillic analytes

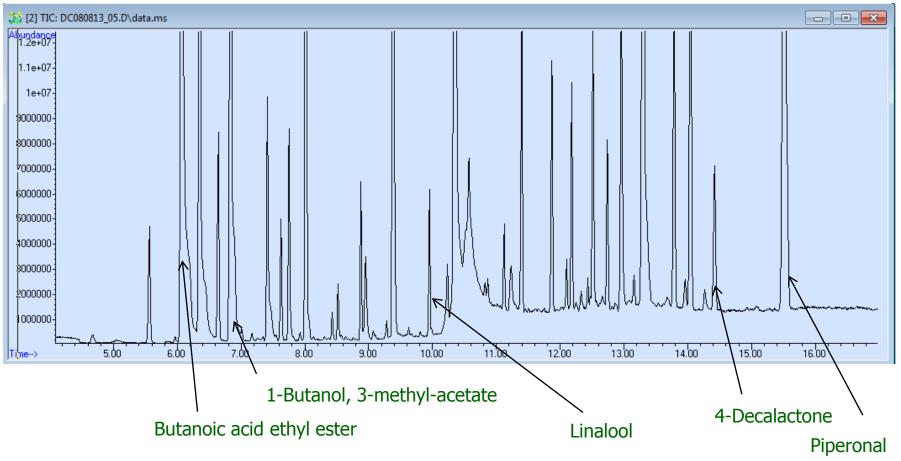








- Frutarom Flavour and ingredients company
- Working with Darren Caven-Quantrill (Flavour Chemistry Manager)
- Full Evaporative Technique (FET) on a number of flavoured drinks
- Current method Manual extraction 2-3 hours











Herbal based Liquor (35% alcohol)

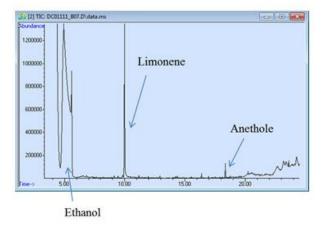


Figure 3(a) Herbal based liquor using Static Headspace

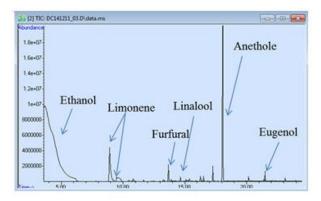


Figure 3 (b) Herbal based liquor using Headspace SPME Static Headspace

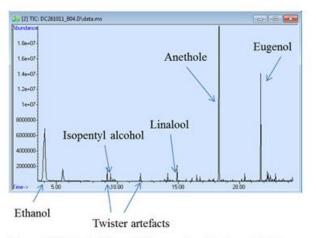


Figure 3(c) Herbal based liquor using Twister SBSE

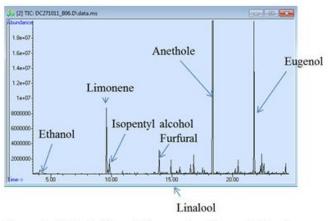


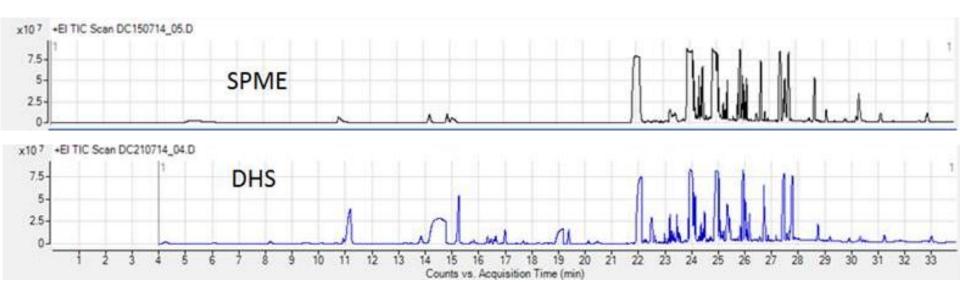
Figure 3 (d) Herbal based liquor using Dynamic Headspace







Method improvement – enriching analytes in distilled spirit









Briefly mention









CF200 (Centrifuge and mixer)

Before centrifugation

1.0		
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	N	













Acknowledgements

- Rick Youngblood
- Ken Brady
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