AUTOMATED HEADSPACE

Headspace Principles

Graham Broadway

Headspace Gas Chromatography

Background to the HS Technique

- *Headspace is a technique suitable for the analysis of volatile or relatively volatile components in difficult sample matrices.*
- *Effectively extracts the components of interest from the sample matrix to give enhanced sensitivity*
- *Prevents contamination of the GC system and column with a non-volatile matix*
- *First commercially available instrument introduced by PerkinElmer in the late 1960's*
- *Initially designed as a technique to analyse **alcohol in blood.***

Headspace Gas Chromatography
What is it ?

- Solvent-free, automated extraction of volatiles from:-
  - Liquids : Aqueous samples, Oils, Emulsions, Gels, Ointments, etc.
  - Solids : Polymers, Resins, Pharmaceutical powders, Soils

COMBINED WITH :-

- Automated direct injection of the headspace volatiles into the analytical column of the GC system.

Headspace Gas Chromatography
When to Use It ?

- When performing qualitative or quantitative analysis of volatiles in difficult sample matrices
- When the entire sample should *not* be injected into the GC
- When minimum sample handling is desirable
- When high sample throughput is required
- Ideal for analysis of trace levels as well as low to medium concentrations of components of interest

Headspace Gas Chromatography
Typical Applications Areas:-

- Pharmaceutical - OVI Analysis
- Food - Flavours & other volatiles
- Beverages - Brewing - Higher Alcohols, Diketones, DMS.
- Beverages - Tea & Coffee, Soft Drinks - Flavours
- Environmental - Soil - BTX & VOC's
- Environmental - Water - BTX & VOC's
- Environmental - Air - BTX & VOC's
- Fragrance & Essential Oils - Profiling and QC monitoring
- Polymers - Monomers & Volatiles
- Packaging Industry - Retained Solvents
- Forensic - Alcohol in Blood *, Solvents in Blood
- Medical & Microbial - Bacteria Profiling, Sutures & Tubing
- Many others …………………………….

* First developed Headspace Application

PerkinElmer Gas Chromatography

Sealed Sample Vial comprising :-

. 22mls glass vial

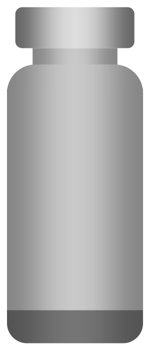
. Aluminium closure

. Aluminium spacer

. Septum

PLUS

Sample



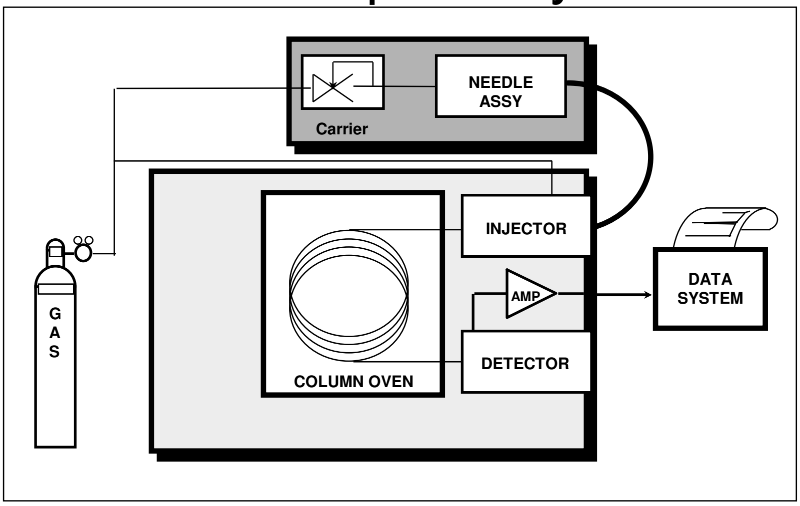
PerkinElmer Gas Chromatography

Thermostat sample vial in heated block until fully equilibrated

Typical Headspace Sampler

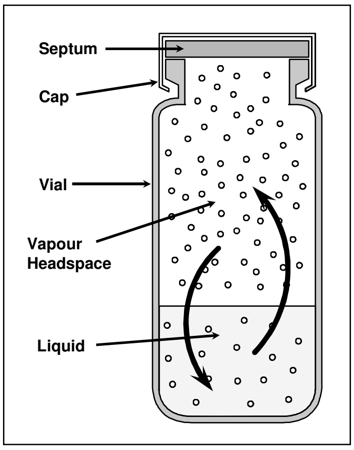
TurboMatrix HS 40

The Headspace System



The Process of Equilibrium

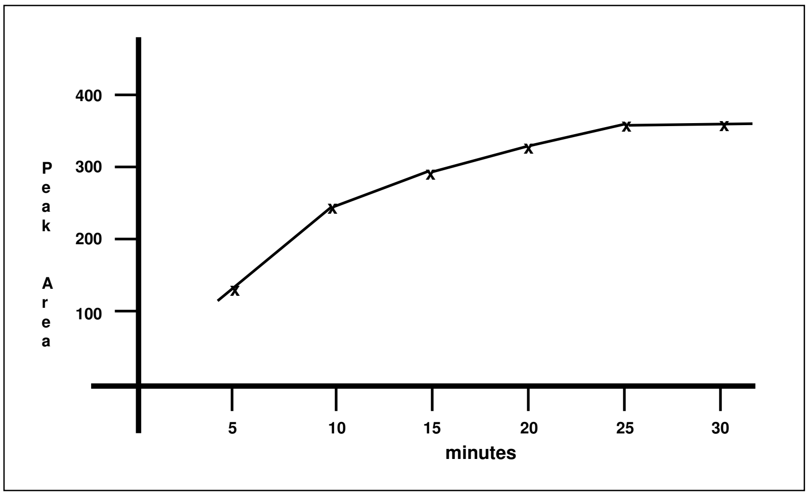
- Sample sealed in closed vial
- Constant temperature conditions maintained
- Volatile sample components leave sample phase and enter gas phase
- Equilibrium is reached when concentrations between liquid and gas phase “balance”
- Headspace gas phase sampled <u>only</u> at equilibrium



Thermostatting Time

- The period during which the sample vial is heated to establish thermodynamic equilibrium
- Time required depends on the volume and viscosity of sample
- The Option of a Shaker can reduce Thermostatting time considerably.

Effect of Thermostatting Time on Peak Areas

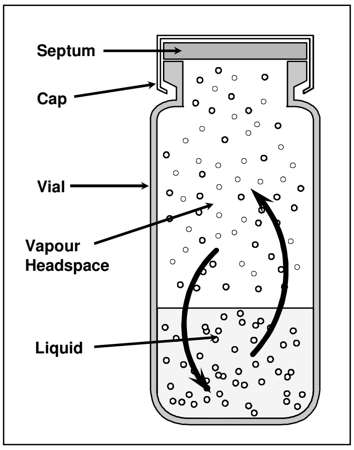


Thermostatting Temperature

- Determined experimentally
- Set high enough to drive sample components of interest from liquid to gas phase
- The gas pressure for sampling MUST be greater than the vapour pressure of the sample
- Thermostatting temperature should not be set above the boiling point of the solvent (liquid) - safety caps will vent sample (non-reproducible results); vials could burst if pressure becomes too great

The Process of Equilibrium

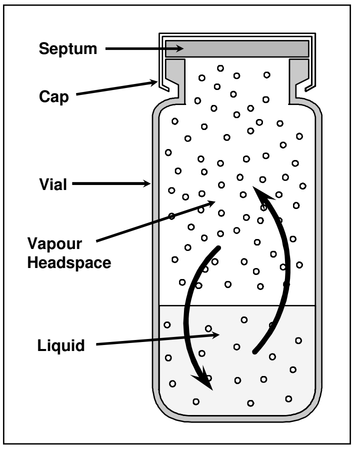
- $K = C_l/C_g$
- High Partition Coefficient



The Process of Equilibrium

- $K = C_l/C_g$

♦Low Partition Coefficient



Partition Coefficients [K] of compounds in water

Compound	40°C	50°C	60°C	70°C	80°C
Dioxane	1618	1002	642	412	288
Ethanol	1355	820	511	328	216
Propan-2-ol	825	479	286	179	117
Butan-1-ol	647	384	238	179	117
Butan-2-one	139.5	109	68.8	47.7	35
Ethyl acetate	62.4	42.7	29.3	21.8	17.5
n-Butyl acetate	31.4	20.6	13.6	9.82	7.25
Benzene	2.90		2.27	1.71	1.66
Toluene	2.82	2.23	1.77	1.49	1.27
o-Xylene	2.44	1.79	1.31	1.01	0.99
Dichloromethane	5.65	4.29	3.31	2.60	2.07
1,1,1-Trichloroethane	1.65	1.53	1.47	1.26	1.18
Tetrachloroethylene	1.48		1.27	0.78	0.87
n-Hexane	0.14	0.068	0.043	0.012	0.0075
Cyclohexane	0.077	0.055	0.040	0.030	0.023

Headspace Gas Chromatography

$$\text{Peak Area} = \frac{C^o}{K + \beta}$$

Where C^o = Original Concentration
K = Partition Coefficient
β = Phase Ratio (V^G/V^S)

Headspace Gas Chromatography

Vol of Vial = 22.3ml

1 ml sample	β = (22.3 - 1) /1 = 21.3
5 ml sample	β = (22.3 - 5) /5 = 3.46

	K (at 60°C)
n-Hexane	0.043
Ethanol	511.0

Headspace Gas Chromatography

If Original Concentration (C^o) = 10ppm

$$\text{Area} = \frac{C^o}{K + \beta}$$

1 ml sample

N-Hexane $= \frac{10}{0.043 + 21.3} = 0.47$ **Ethanol** $= \frac{10}{511 + 21.3} = 0.019$

Headspace Gas Chromatography

If Original Concentration (C^o) = 10ppm

$$\text{Area} = \frac{C^o}{K + \beta}$$

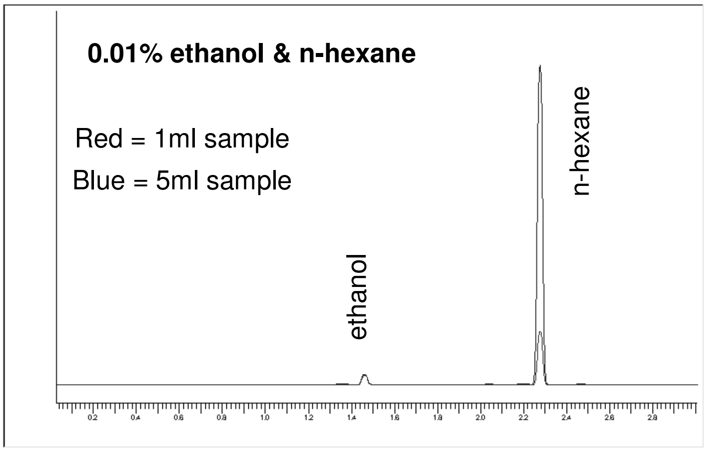
1 ml sample

N-Hexane $= \frac{10}{0.043 + 21.3} = 0.47$ **Ethanol** $= \frac{10}{511 + 21.3} = 0.019$

5 ml sample

N-Hexane $= \frac{10}{0.043 + 3.46} = 2.85$ **Ethanol** $= \frac{10}{511 + 3.46} = 0.019$

Effect of Sample Volume and Partition Coefficient on Peak Area



Affect of Partition Coefficient and Phase Ratio on Peak size

	Volume of Sample (mL)								
K	0.001	0.01	0.03	0.1	0.2	1	5	10	15
0.0001	1.0	10.0	30.0	100.0	200.0	1000.0	4999.9	9999.2	14997.0
0.001	1.0	10.0	30.0	100.0	200.0	1000.0	4998.6	9991.9	14969.7
0.01	1.0	10.0	30.0	100.0	200.0	999.5	4985.7	9920.0	14702.0
0.1	1.0	10.0	30.0	100.0	199.8	995.3	4860.3	9253.7	12471.9
1	1.0	10.0	30.0	99.6	198.2	955.4	3883.9	5535.7	4955.4
5	1.0	10.0	29.8	97.8	191.4	810.6	2051.9	1987.2	1347.1
10	1.0	10.0	29.6	95.7	183.5	681.5	1290.8	1103.2	705.2
50	1.0	9.8	28.1	81.7	137.9	299.7	325.4	242.0	146.6
100	1.0	9.6	26.5	69.0	105.2	176.3	168.1	122.5	73.6
200	1.0	9.2	23.7	52.7	71.4	96.7	85.5	61.6	36.9
500	1.0	8.2	18.0	30.8	36.3	41.0	34.6	24.7	14.8
1000	1.0	6.9	12.8	18.2	20.0	21.0	17.3	12.4	7.4
10000	0.7	1.8	2.1	2.2	2.2	2.1	1.7	1.2	0.7

Headspace Gas Chromatography
Salt Addition

Effect of adding salts to aqueous samples at 60°C

Salt	Increase in Ethanol Peak Area
Ammonium sulfate	X5
Sodium chloride	X3
Potassium carbonate	X8
Ammonium chloride	X2
Sodium citrate	X5

Headspace Gas Chromatography
Salt Addition

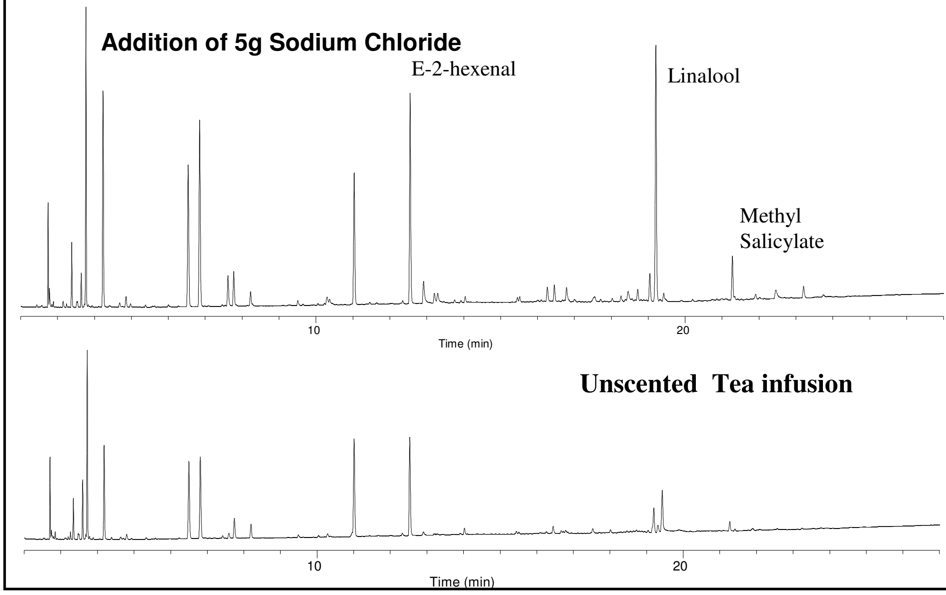
Effect of Sample Volume and Salt Addition (NaCl)

Enrichment Factors at 10 µg/L

	5 mL No Salt	**5 mL Salt**	**10 mL No Salt**	**10 mL Salt**
Dichloromethane	1.00	1.68	1.33	2.97
Trichloroethane	1.00	1.28	1.38	3.55
Trichloroethylene	1.00	1.38	1.74	3.64
Tetrachloroethylene	1.00	1.25	1.90	3.30
Benzene	1.00	1.60	1.53	3.73
Toluene	1.00	1.66	1.79	4.36
Ethylbenzene	1.00	1.68	1.79	4.62
p- and m-xylenes	1.00	1.60	1.76	4.58

Headspace Gas Chromatography

Salt Addition

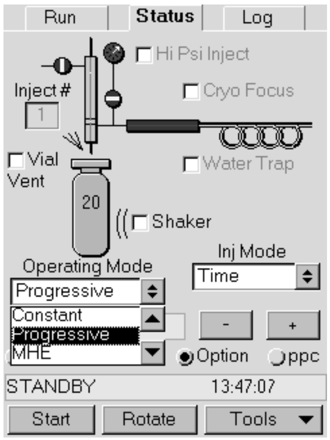


Typical Headspace Sampler

TurboMatrix HS 40

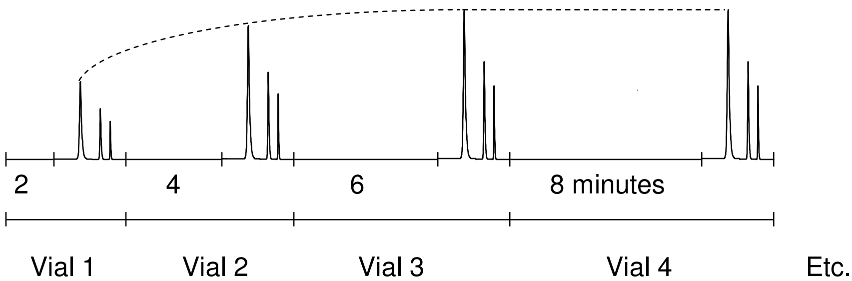
Operating Modes

- *Constant Mode*
  - *Maximum through-put using overlapping thermostatting*
- *Progressive Mode*
  - *Labour saving method development*
- *Multiple Headspace Extraction*
  - *Used for total extraction of volatiles*



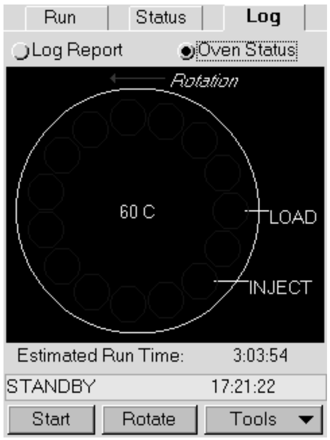
Flexibility
Progressive Mode
Labor saving method development tool

- *Vials with identical contents are analysed*
- *The thermostatting time is automatically increased for each vial*
- *The thermostatting time needed to reach equilibrium is determined*

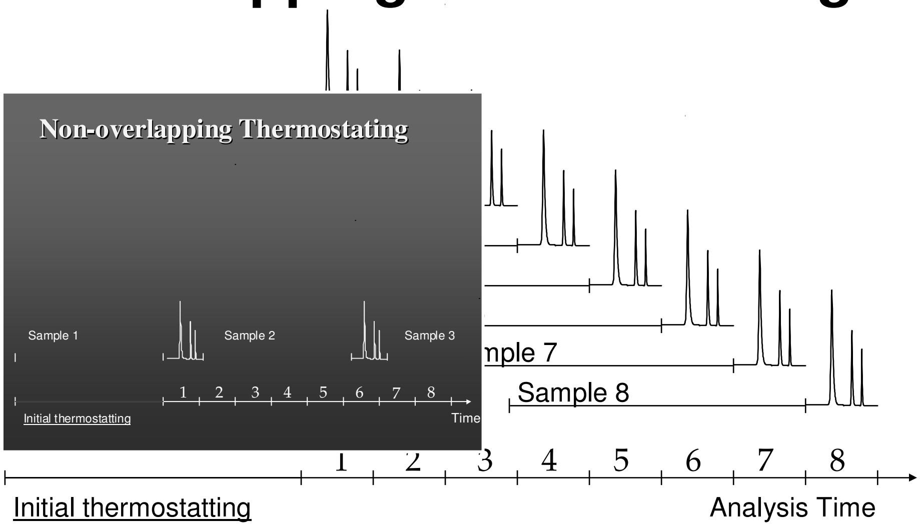


Maximum Sample Throughput

- *Overlapping Thermostatting*
  - *15 position vial ovens can accommodate up to 12 simultaneously thermostatting vials*

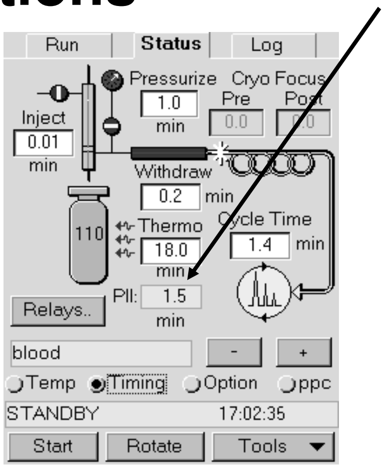


Overlapping Thermostatting

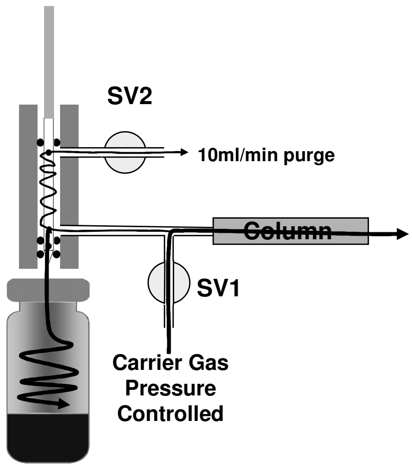


Time Saving PII Calculations

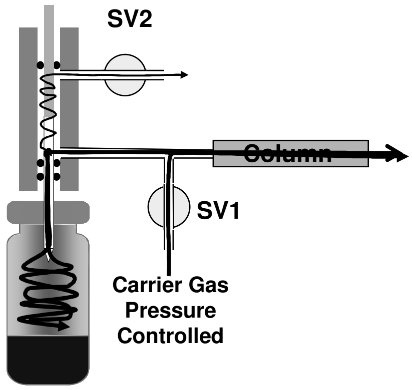
- ***Period from Injection to Injection (PII)***
  - *Automatically calculated for optimum sample throughput*
  - *See the effects of your analysis timing changes on vial throughput*



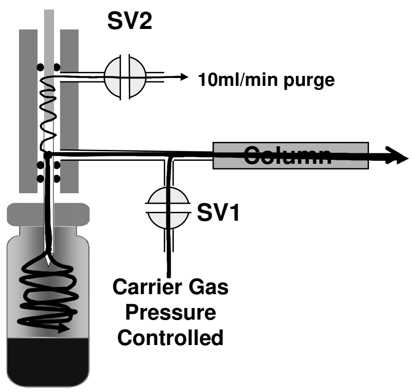
Headspace Pressurisation



Headspace Injection

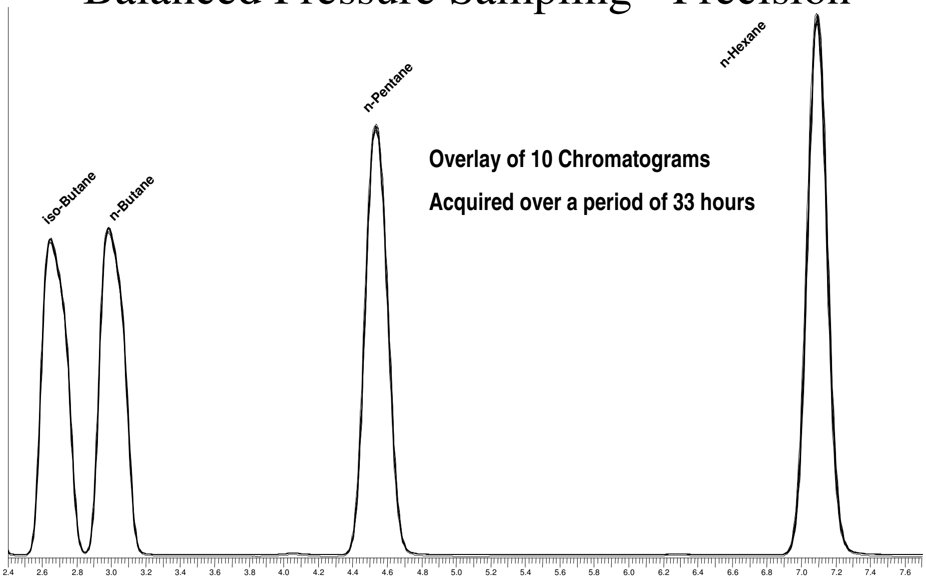


Headspace Standby



Headspace Gas Chromatography

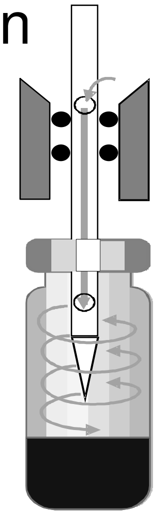
Balanced Pressure Sampling - Precision



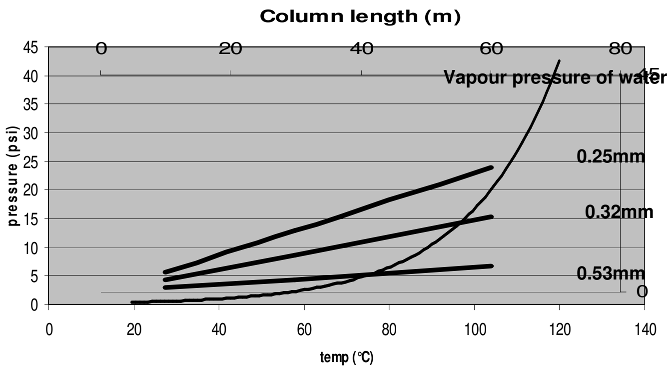
Needle Assembly

Vial Pressurisation

- Sample vial is filled (pressurised) with carrier gas
- PRESSURISATION TIME is the period allowed for the carrier gas to fill the vial.
- A 1.0 minute minimum Pressurisation Time is recommended to fill the vial completely, and stabilise the pressure to match the column pressure. (3 minutes would be better)



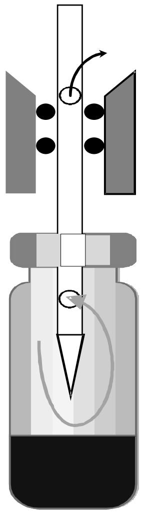
Inlet Pressure of Capillary Columns



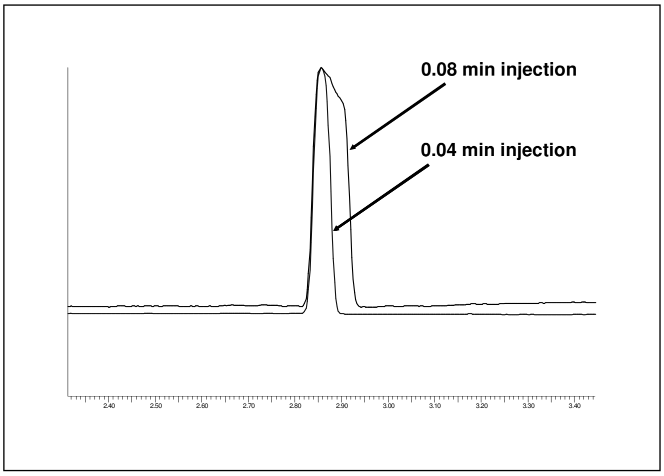
Inlet pressures for Helium at 25cm/s at 100°C
Vapour pressure with respect to temperature

Sample Injection

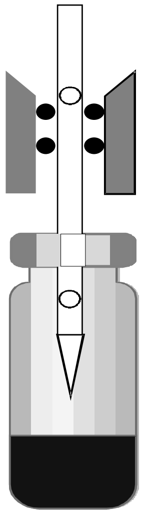
- INJECTION TIME is the period in which the sample (gas phase) is drawn from the vial and injected onto the column
- The length of time determines the volume of gas (and sample vapour) transferred onto the column
- Default injection time is 0.08 minutes (or 4.8 seconds).
- 0.04 minutes (2.4 seconds) is more suitable for capillary chromatography.



Effect of Injection Time

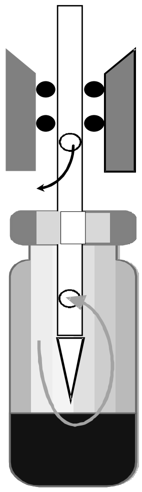


Sample Injection



- WITHDRAWAL TIME is the period in which the needle will continue to remain in the sample vial after the Injection Time has elapsed
- Allows sample to be carried well onto the first part of the column
- Withdrawing needle from vial too early causes a small pressure drop along the capillary transfer line - default withdrawal time is 0.2 minutes (12 seconds)

Sample Injection



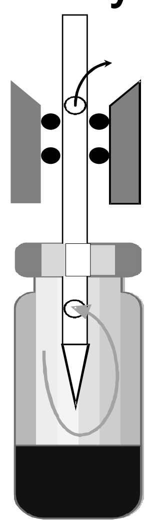
- VENT TIME is the period in which the pressure in the sample vial is vented to atmosphere
- Should be long enough to allow vial to depressurise (0.2 - 0.3 minutes)
- Venting is turned off only for toxic samples
- Depressurisation makes vial safe for disposal

Factors affecting Sensitivity

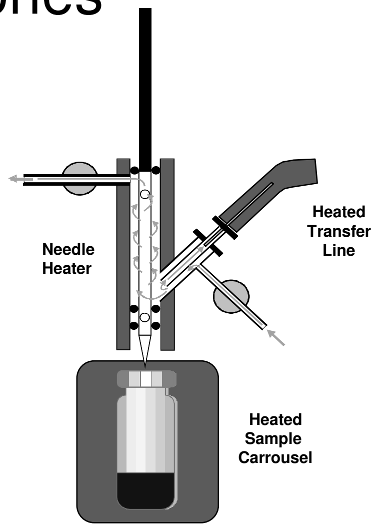
- Partition Coefficient
  - Compounds with low Partition Coefficients will have greater sensitivity
- Temperature
  - Higher temperature will reduce the Partition Coefficient
- Volume of Sample in Vial
  - Has greater effect for compounds with low Partition Coefficients

Factors affecting Sensitivity

- INJECTION TIME The longer the injection time the greater the amount of sample transferred to the column
- The longer the injection time the broader the peak
- The higher the carrier gas flow the greater the amount of sample transferred to the column
- Large diameter columns operate at higher carrier gas flows.

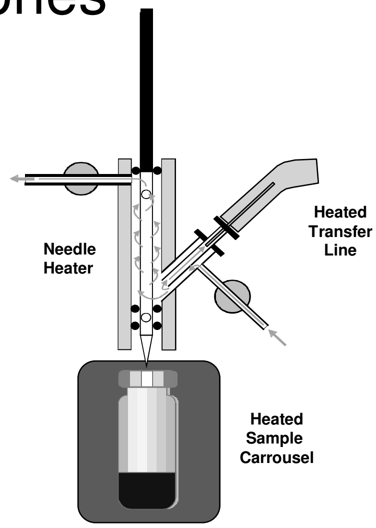


Heated Zones



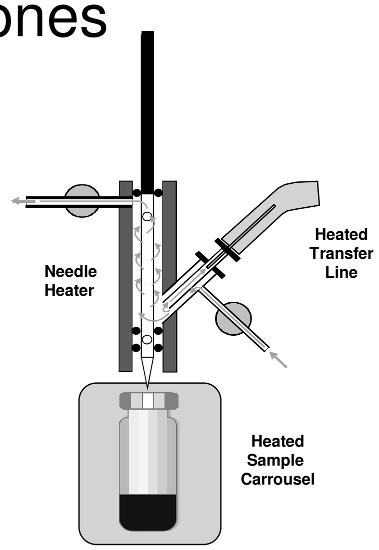
Heated Zones

- OVEN TEMPERATURE (sample)



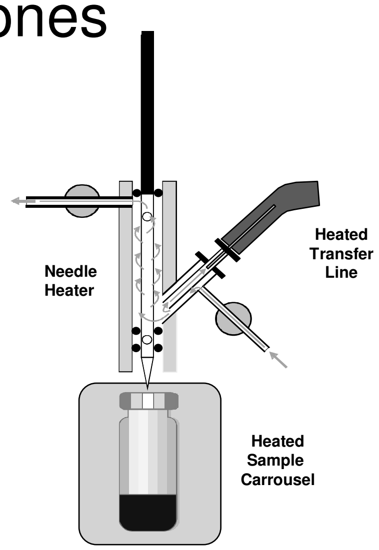
Heated Zones

- OVEN TEMPERATURE (sample)
- NEEDLE TEMPERATURE



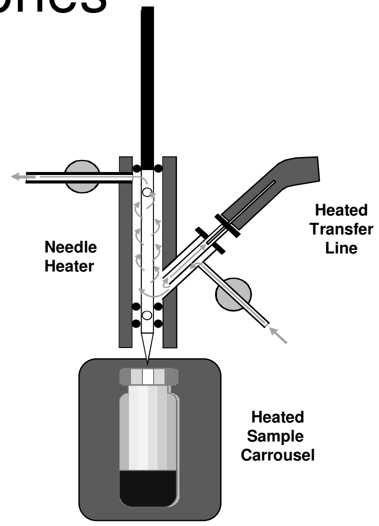
Heated Zones

- OVEN TEMPERATURE (sample)
- NEEDLE TEMPERATURE
- TRANSFER LINE TEMPERATURE



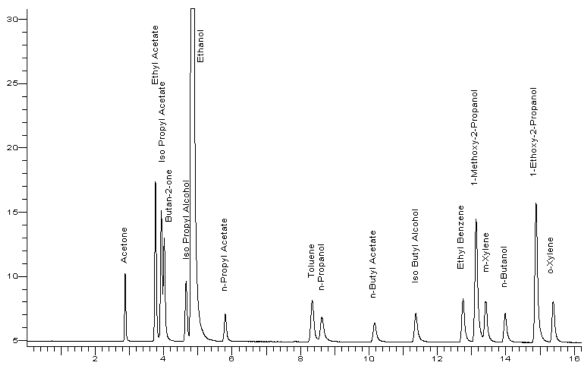
Heated Zones

- OVEN TEMPERATURE (sample)
- NEEDLE TEMPERATURE
- TRANSFER LINE TEMPERATURE
- Heated zones prevent sample condensation prior to reaching the column



Residual Solvents in Printed Film

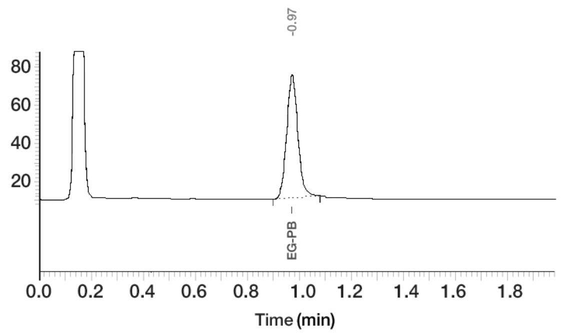
HS Conditions	
Sample:	**10 x 10 cm**
Thermostat Temp:	**70ºC**
Thermostat Time:	**60 min**
Sampling Time:	**0.05 min**



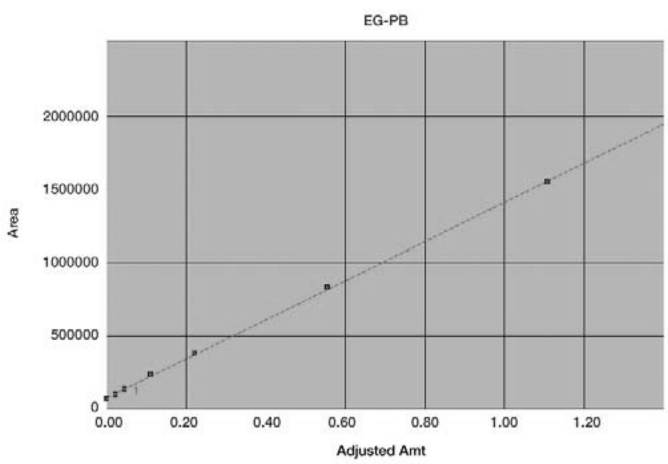
Ethylene Glycol in Used Engine Oil

HS Conditions

Sample:	**100µL + 5mg derivatising agent**
Thermostat Temp:	**120ºC**
Thermostat Time:	**18 min**
Sampling Time:	**0.01 min**
Time between injections:	**3 min**



Linearity of Ethylene Glycol Calibration

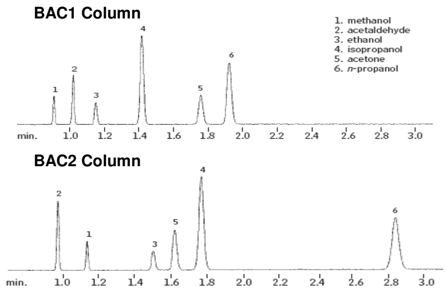


Blood Alcohol Analysis

HS Conditions

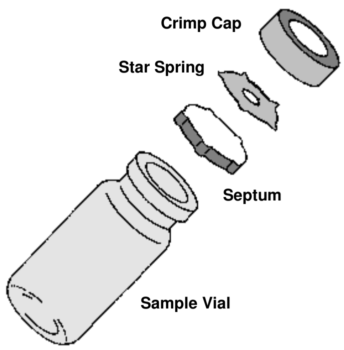
Sample:	**1 ml**
Thermostat Temp:	**70ºC**
Thermostat Time:	**15 min**
Sampling Time:	**0.01 min**
Time between injections:	**4 min**

Methanol	**0.1%**
Acetaldehyde	**0.2%**
Ethanol	**0.2%**
iso-Propanol	**0.1%**
Acetone	**0.01%**
n-Propanol	**0.1%**



Vial Caps and Septa

- CAPS AND SEPTA
- Patented Perkin-Elmer safety system consists of:
  - aluminium crimp cap
  - aluminium star spring
  - septa
- System acts as a safety release and vents if internal pressure becomes too great (> 5 to 6 Bar)



Septa Available

- Butyl Rubber
  - Cheap. Not suitable for trace analysis. Max Temp 100°C
- Butyl Rubber with PTFE film
  - Max Temp 100°C
- Silicone Rubber with PTFE film
  - High Purity septa for trace analysis. Max Temp 210°C
- Silicone Rubber with Aluminium film
  - High Purity septa for trace analysis. Max temp 120°C

Sources of Contamination

- Vials, caps, and closures are all possible sources of sample contamination
- Reseal all open bags of septa, and store open boxes of vials in a clean, dry place away from all possible contaminants
- Always cap vials near a clean air source to avoid contaminants in the air from being trapped in with sample
- Discard aluminium caps or star springs that are bent, dented or have wrinkles

AUTOMATED HEADSPACE

Headspace Calibration

Calibration

- **External standard**
  - **Most commonly used technique**
- **Internal standard**
  - **Limited use because of partition coefficient effects**
  - **Often used to check HS-GC System**
- **Total vaporisation**
- **Standard addition**
- **MHE**

Classification of Liquid samples

- In a simple sample where one component is present in solution of a single component matrix the partition coefficient will remain constant at a given temperature.
- When the matrix consists of 2 or more components the partition coefficient will be affected by the relative concentrations of each component.
- The Activity coefficient (γ) will influence the concentration of each component in the gas phase

Classification of Liquid Samples

Ideal Dilute Solution

$$\gamma = \text{constant}$$

$$p' = \gamma . x . p^o$$

$$A = CF.x$$

p' = partial vapour pressure
= gas phase concentration
γ = activity coefficient (matrix effect)
X = conc. In the liquid sample
P^o = vapour pressure of the pure compound at temperature T
CF = calibration factor (constant)
A = Peak area

Classification of Liquid Samples

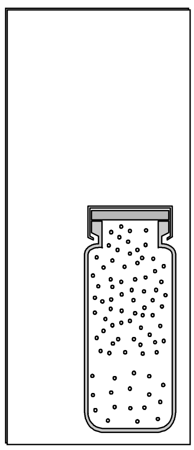
Pure Matrix Available	$\gamma_{sample} = \gamma_{standard}$
Eg Impurities in used engine oil	**External Standard**
Standardised Matrix	$\gamma_{sample} \sim \gamma_{standard}$
Saturation of an aqueous solution with salt	**External Standard**
Simulated matrix	$\gamma_{sample} \sim \gamma_{standard}$
Analysis of flavours in Spirits by calibration in 40% ethanol soln.	**External Standard**
Unknown matrix	$\gamma_{sample} \neq \gamma_{standard}$
Eg Shampoo	**Standard addition**
Solid Samples	
Eg Food packaging	**Total Vaporisation**

Total Vaporisation

Standard Injected directly into vial

Vial heated & Standard vaporises

Total vaporisation technique is possible for components up to boiling points of about 300℃

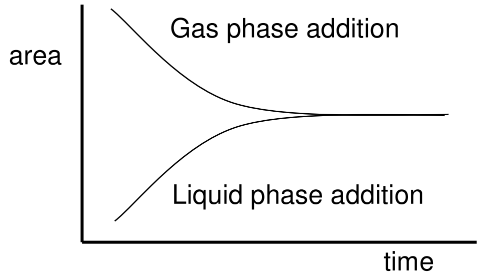


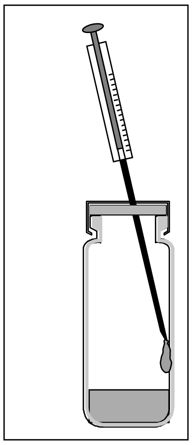
Standard Addition

Standard Injected directly into vial

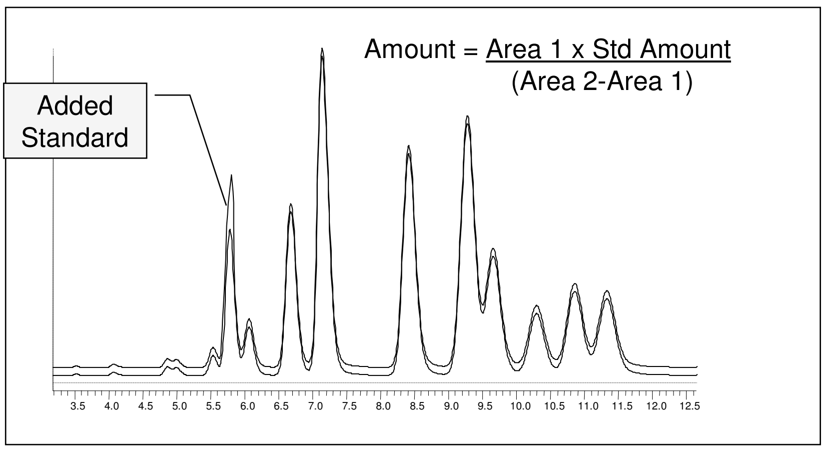
Standard may be added to either the gas phase or liquid phase

Must wait until equilibration is reached





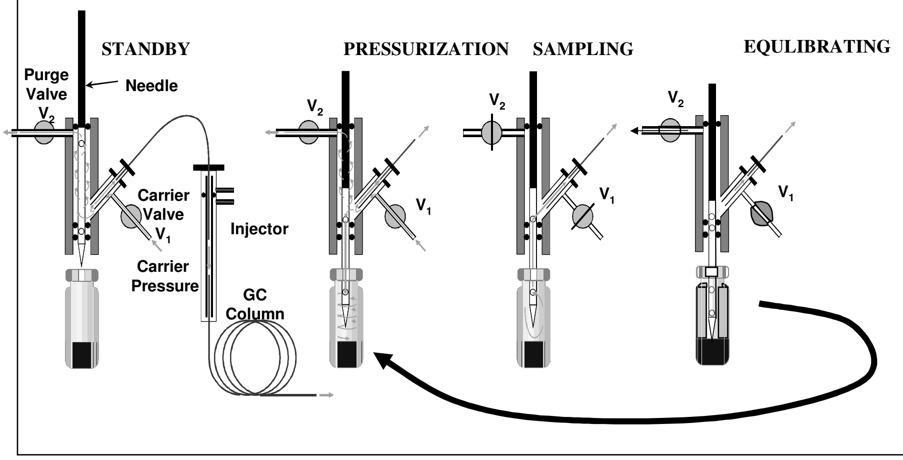
Standard Addition



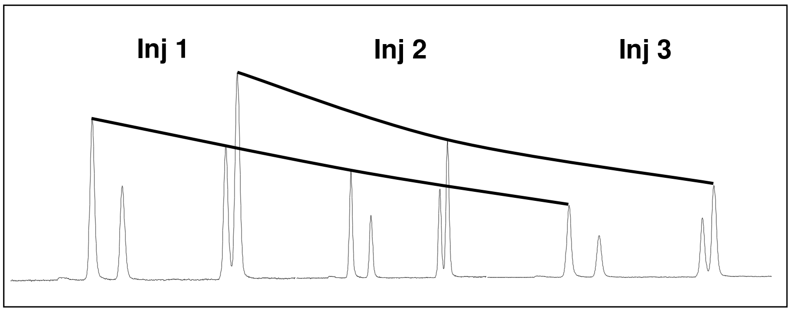
AUTOMATED HEADSPACE

Multiple Headspace Extraction

Multiple Headspace Extraction



Multiple Headspace Extraction



The Peak Area from successive injections Decreases exponentially

Multiple Headspace Extraction

MHE works best for compounds with partition coefficients between 5 and 150 (approx)

Multiple Headspace Extraction Calculations

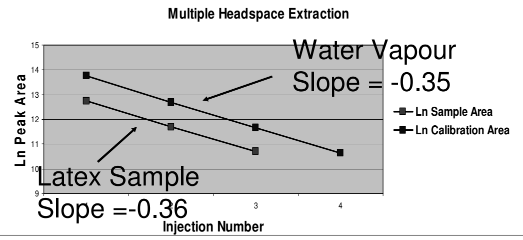
Calculates the area from Total extraction of the volatiles from the sample matrix

$$\Sigma A = \frac{A_1}{1 - e^{-k}}$$

$$k = \ln \frac{A_1}{A_2}$$

Determination of Water in Latex by MHE

	Sample Area	Ln Sample Area	Calibration Area	Ln Calibration Area
1	342146	12.74299	953163	13.76754121
2	120712	11.70116	323480	12.68689257
3	44935	10.71297	114840	11.65129513
4			42217	10.65057826



Determination of Water in Latex by MHE

At 150°C it can be assumed that the total water content is vaporised and the MHE procedure will give the same result as a simple headspace method with external standard calibration

The latex was sliced and thermostatted at 150°C for 30 minutes

Result from 3 stage MHE	**0.73% water**
Simple headspace	**0.70% water**

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Simple headspace	**0.70% water**

Multiple Headspace Extraction of Solid Samples

- Solids are not suitable for MHE because of non-linear adsorption isotherms
- Will require extraction with a suitable solvent first

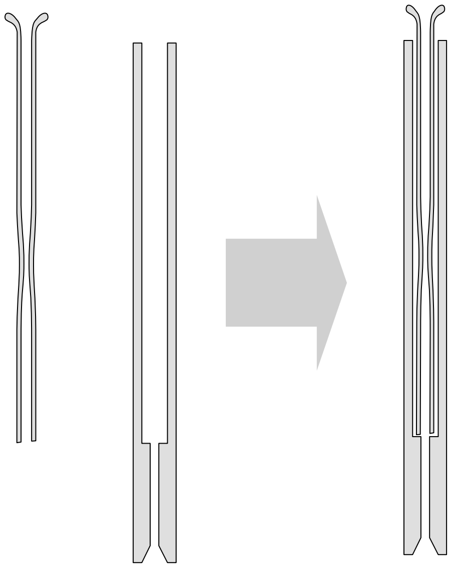
Multiple Headspace Extraction Solid Samples

- **Add a solvent to generate a partition system**
- **Use a polar solvent for a hydrophilic matrices**
  - **Water**
  - **Alcohols**
  - **Glycols**
- **Use a non-polar solvent for hydrophobic matrices**
  - **Benzyl alcohol**
  - **Benzoic acid benzyl ester**
  - **Dimethyl formamide (DMF)**

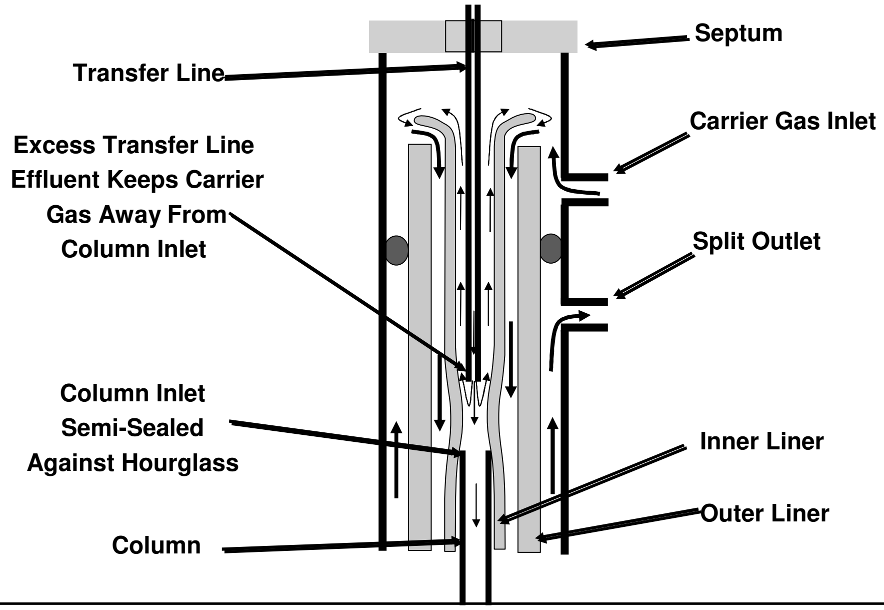
NOTE: - The desorption time often exceeds the equilibration time

Headspace Gas Chromatography
Zero-Dilution Liner (ZDL)

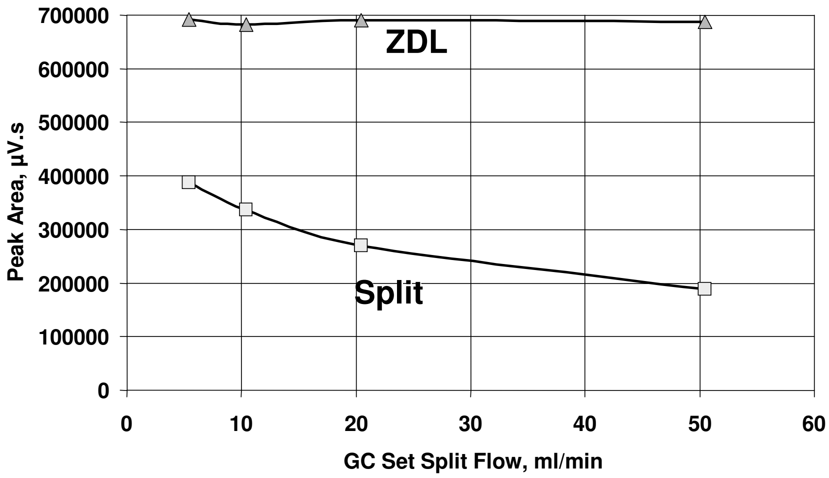
- 2-Piece Design
- Fits inside standard split injector



Headspace Gas Chromatography
ZDL - Principle of Operation



Effect of Split Flow on Peak Area



HS Trap

HS Trap

- **Conventional Headspace suffers because only a small portion of the "headspace" can be injected into the column.**
- **Long inject times cannot be used with high efficiency capillary columns**
- **Can often achieve ppb levels but sub-ppb cannot be achieved with conventional headspace.**

HS Trap

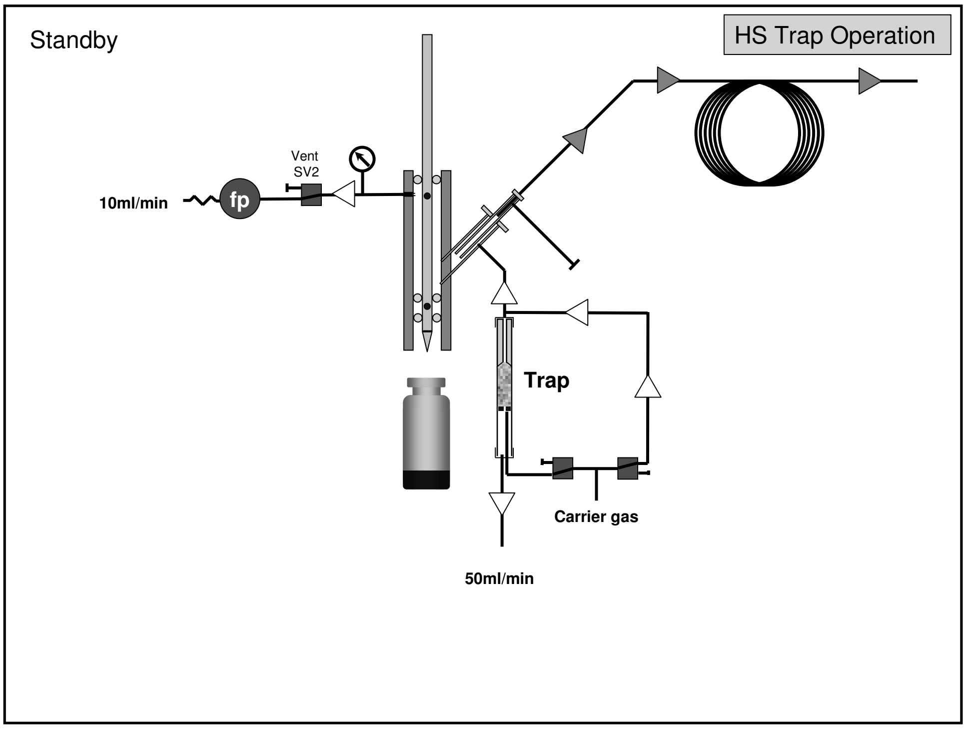
- By incorporating an adsorbent trap detection limits can be improved considerably
- All of the gas phase is vented through a trap
- May be pressurised and vented a number of times
- Amount transferred depends on Pressure of gas in vial

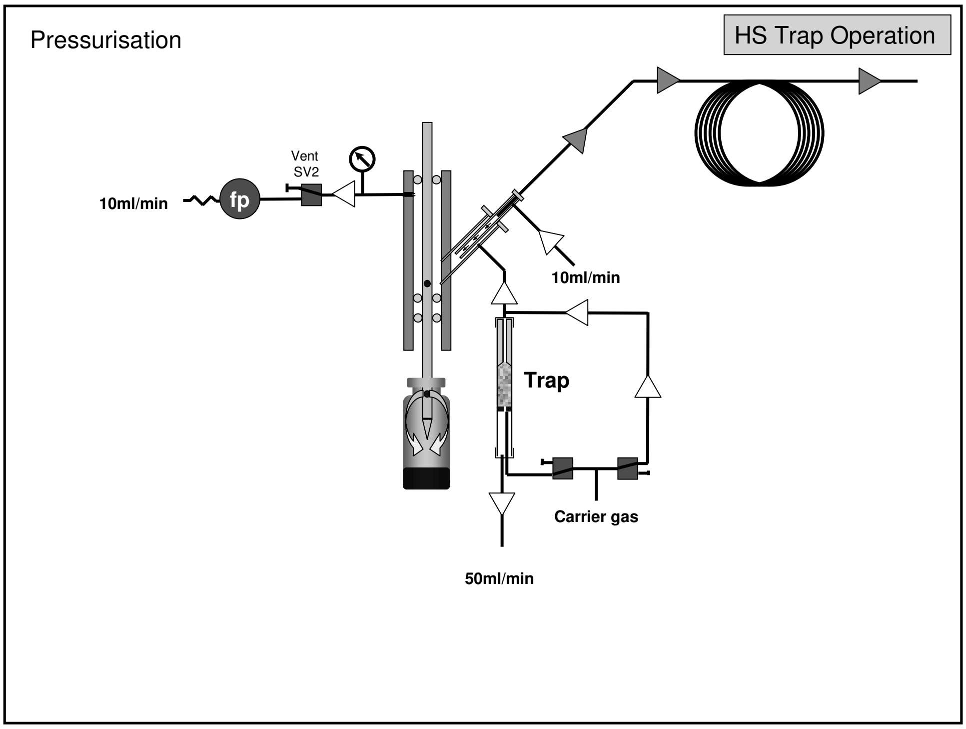
HS Trap
Amount transferred to Trap

Vial P (psig)	% extracted 1 cycle	% extracted 2 cycles	% extracted 3 cycles	% extracted 4 cycles
5	25.38	44.32	58.45	69.00
10	40.49	64.58	78.92	87.45
15	50.51	75.50	87.87	94.00
20	57.64	82.05	92.40	96.78
25	62.97	86.29	94.92	98.12
30	67.11	89.19	96.44	98.83
35	70.42	91.25	97.41	99.23
40	73.13	92.78	98.06	99.48
45	75.38	93.94	98.51	99.63
50	77.28	94.84	98.83	99.73
55	78.91	95.55	99.06	99.80
60	80.32	96.13	99.24	99.85

HS Trap

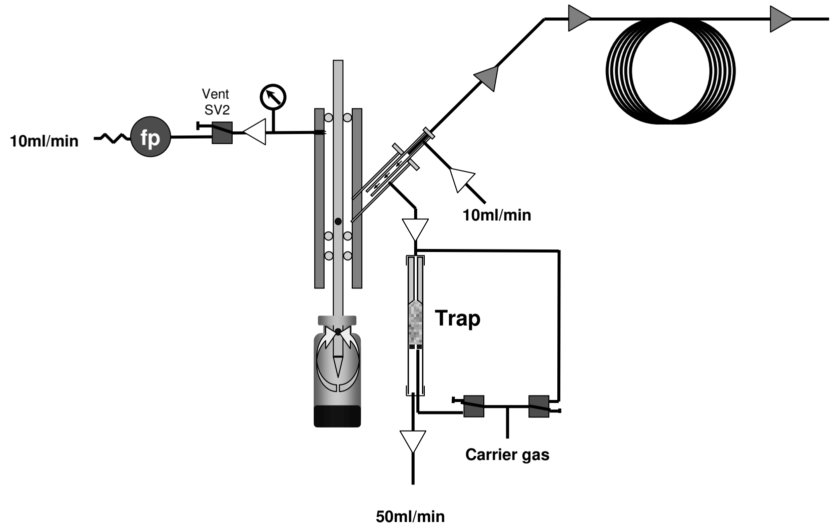
- Usual Trap consists of 2 carbon based adsorbents
- Both adsorbents are hydrophobic
- Capable of trapping compounds such as chloromethane, vinyl chloride and freon 12 at 35°C
- At 35°C water can be purged from trap
- Trap is then heated at 40degC/s to transfer adsorbed components to column


Standby
HS Trap Operation
Vent
SV2
10ml/min
fp
Trap
Carrier gas
50ml/min


Pressurisation
HS Trap Operation
Vent
SV2
10ml/min
fp
10ml/min
Trap
Carrier gas
50ml/min

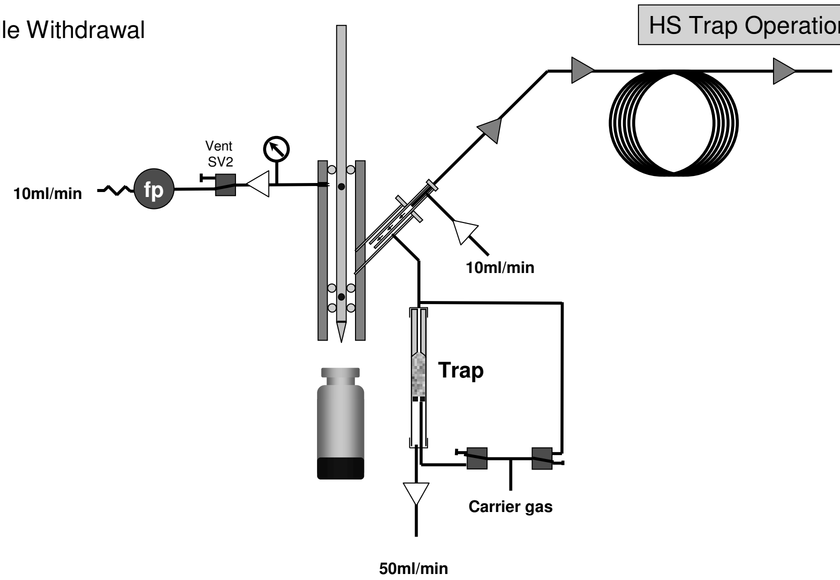
Trap Load

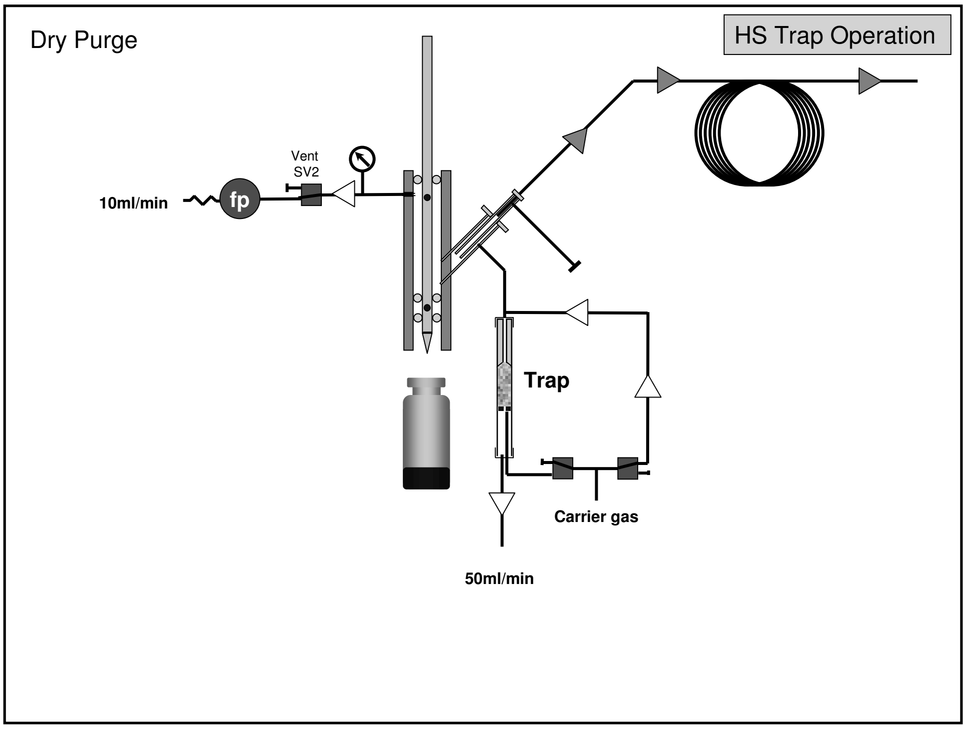
HS Trap Operation

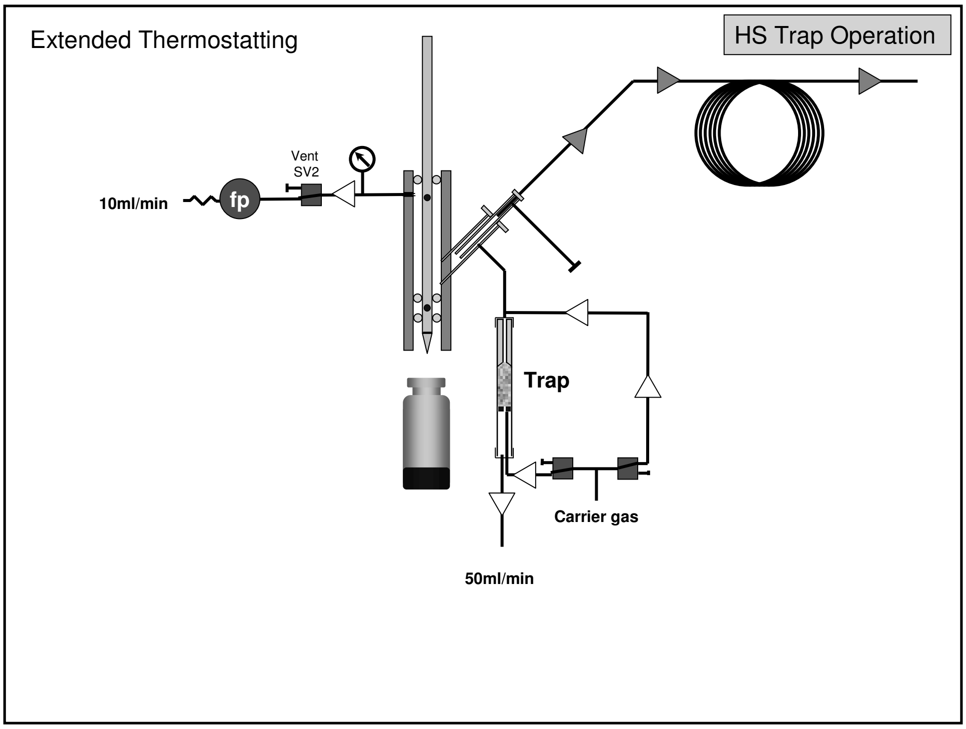


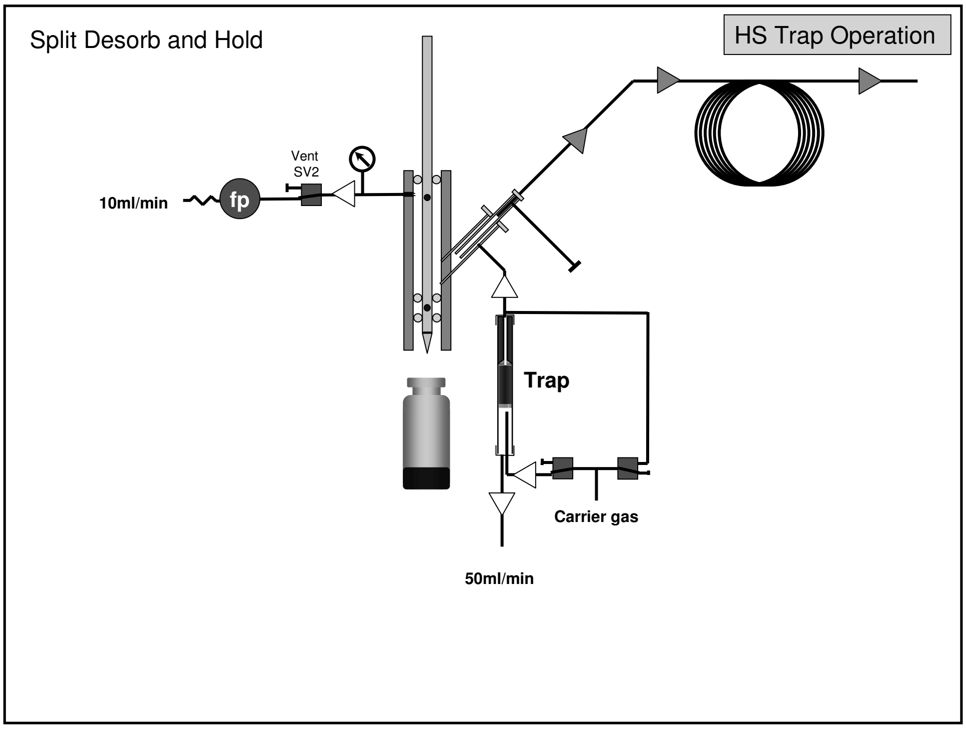
Needle Withdrawal

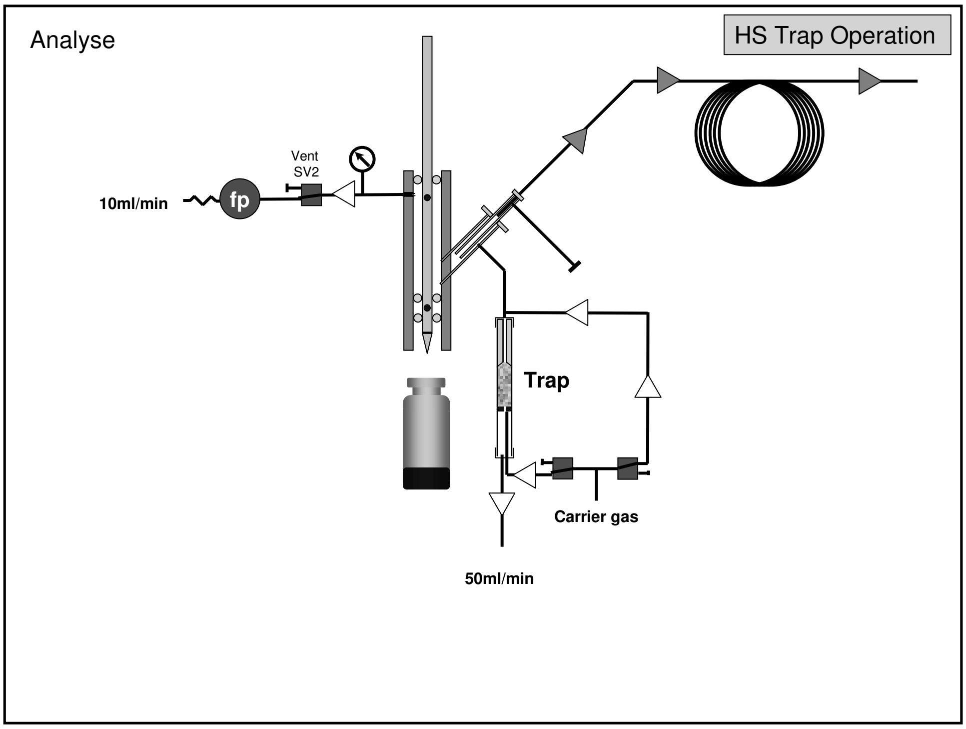
HS Trap Operation




Dry Purge
HS Trap Operation
Vent
SV2
10ml/min
fp
Trap
Carrier gas
50ml/min


Extended Thermostatting
HS Trap Operation
Vent
SV2
10ml/min
fp
Trap
Carrier gas
50ml/min


Split Desorb and Hold
HS Trap Operation
Vent
SV2
10ml/min
fp
Trap
Carrier gas
50ml/min


Analyse
HS Trap Operation
Vent
SV2
10ml/min
fp
Trap
Carrier gas
50ml/min

GC-MS

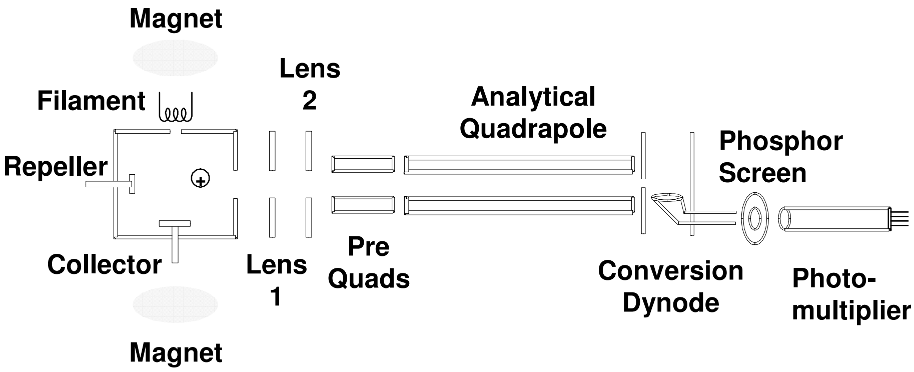
- A Mass Spectrometer provides positive identification of components eluting from the GC Column.
- May also act as a selective detector
- By monitoring selected ions can improve detection limits

Clarus GC-MS Systems

• Mass Range	2 – 1200 Daltons
• Scan Speed	up to 6200 Daltons/s
• High Speed Turbomolecular pump	255L/s

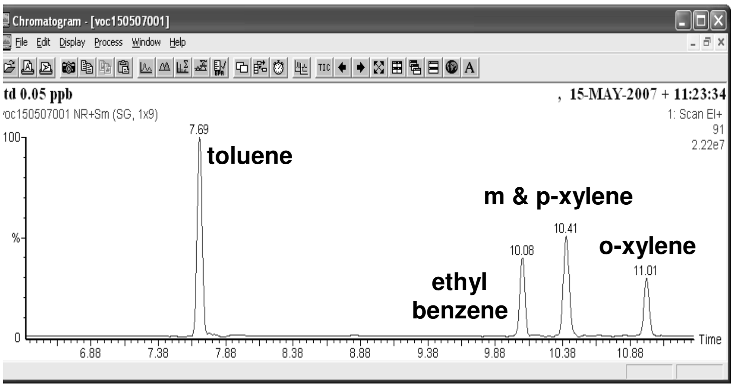
  – Carrier gas flows as high as 5ml/min so can be used with 0.53mm diameter columns

Ion Optics

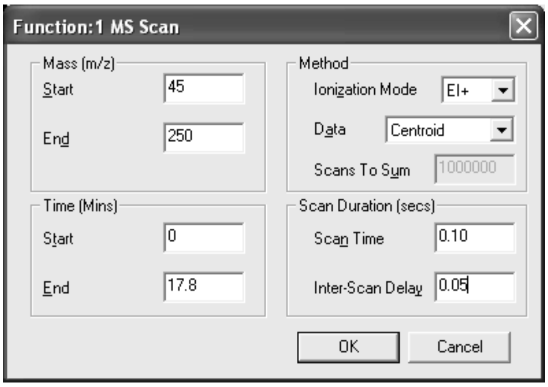


0.05ppb Toluene and Xylenes in Water using HS Trap

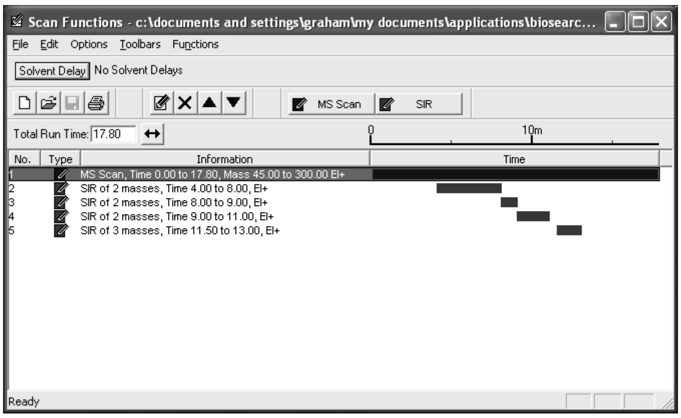
Chromatogram of m/z 91 taken from a full scan from m/z 45 to 250 with a scan time of 0.1s and inter-scan delay of 0.05s



MS Method
Full Scan Mode

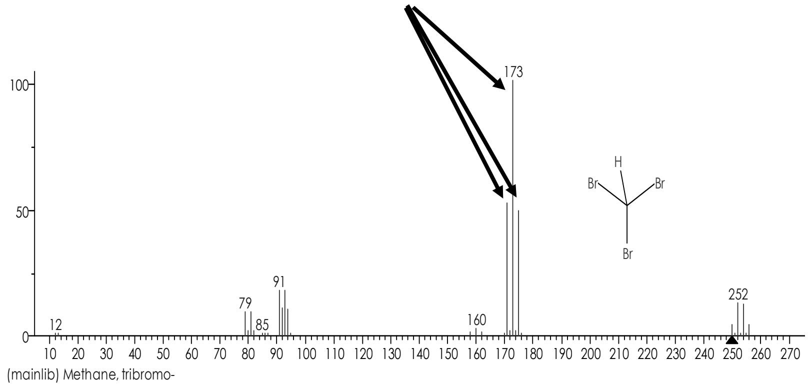


MS Method
Multiple Functions

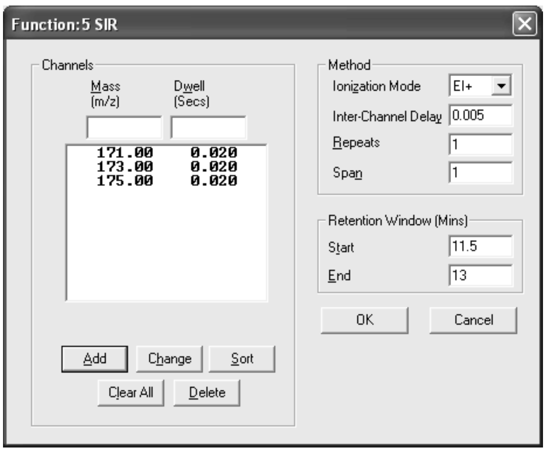


Mass Spectrum of Tribromomethane

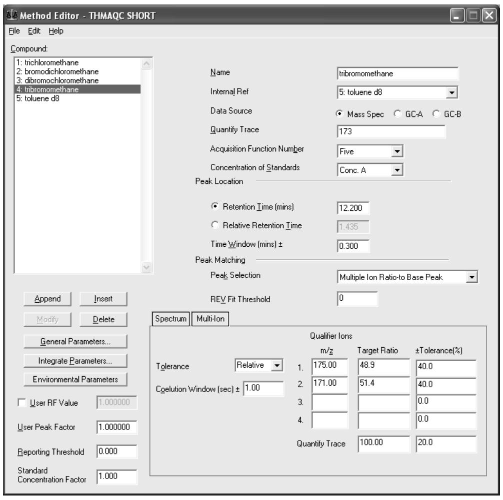
Use m/z 173 for quantitation
m/z 171 and 175 as qualifier ions



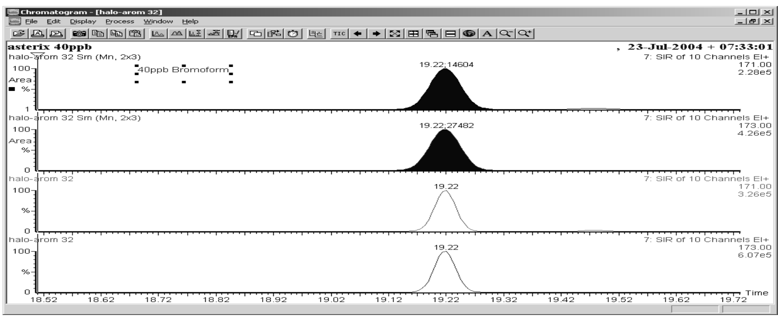
MS Method Function 5 SIR Mode



Quantitative Method

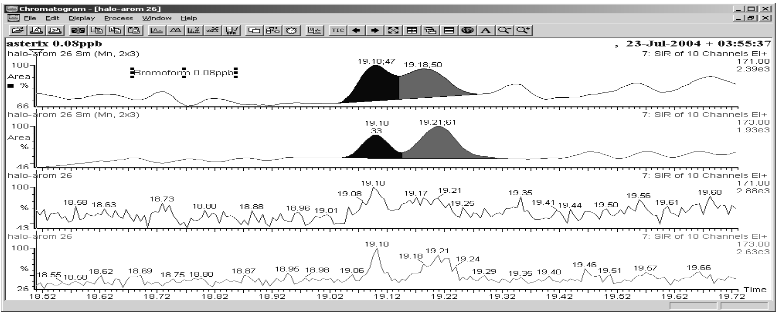


Tribromomethane 40ppb



- m/z 171=53.1% m/z 173
  - Pass Qualifier ion tolerance

Tribromomethane 0.08ppb



- m/z 171=82% m/z 173
  - Fail Qualifier ion tolerance