Comprehensive Analysis of Residual Solvents in Water-Soluble Articles Using Dynamic Headspace

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Background

- ICH (Q3C) lists 61compounds as residual solvent impurities classified by toxicity
  - Class 1 – should be avoided
  - Class 2 – should be limited
  - Class 3 – can be assayed by non-specific techniques – LOD
- USP <467> compendial method
- Selectivity– no single analytical column
- Analyte confirmation – sometimes need multiple columns or MSD
- Sensitivity - Static headspace

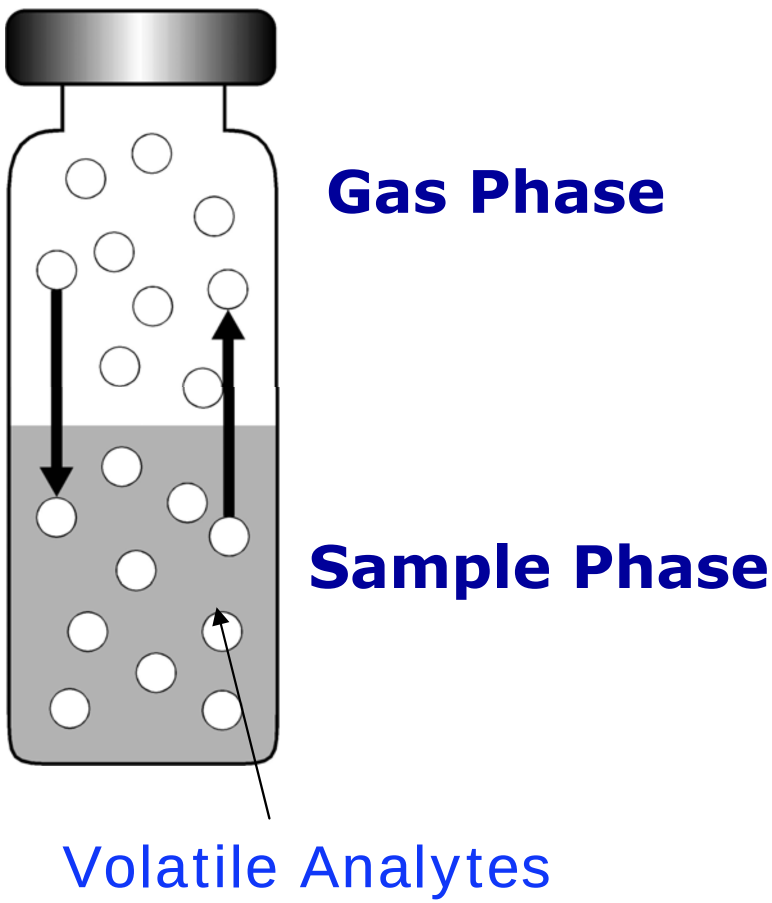
  Great fit for application, not exhaustive or ideally sensitive

Scope

1. Example of Water Soluble Articles
2. Advantages of Dynamic Headspace (Sensitivity)
    1. Added sensitivity brings opportunity
    2. More representative sample
3. Comprehensive dual column FID analysis using Modulated Accelerated Column Heating (Selectivity and Confirmation)
    1. Enhance Selectivity – confirmation analysis
    2. Enhance Sensitivity - LOD
    3. Linearity & Range
    4. Reproducibility
    5. Time Saving

Headspace Analysis

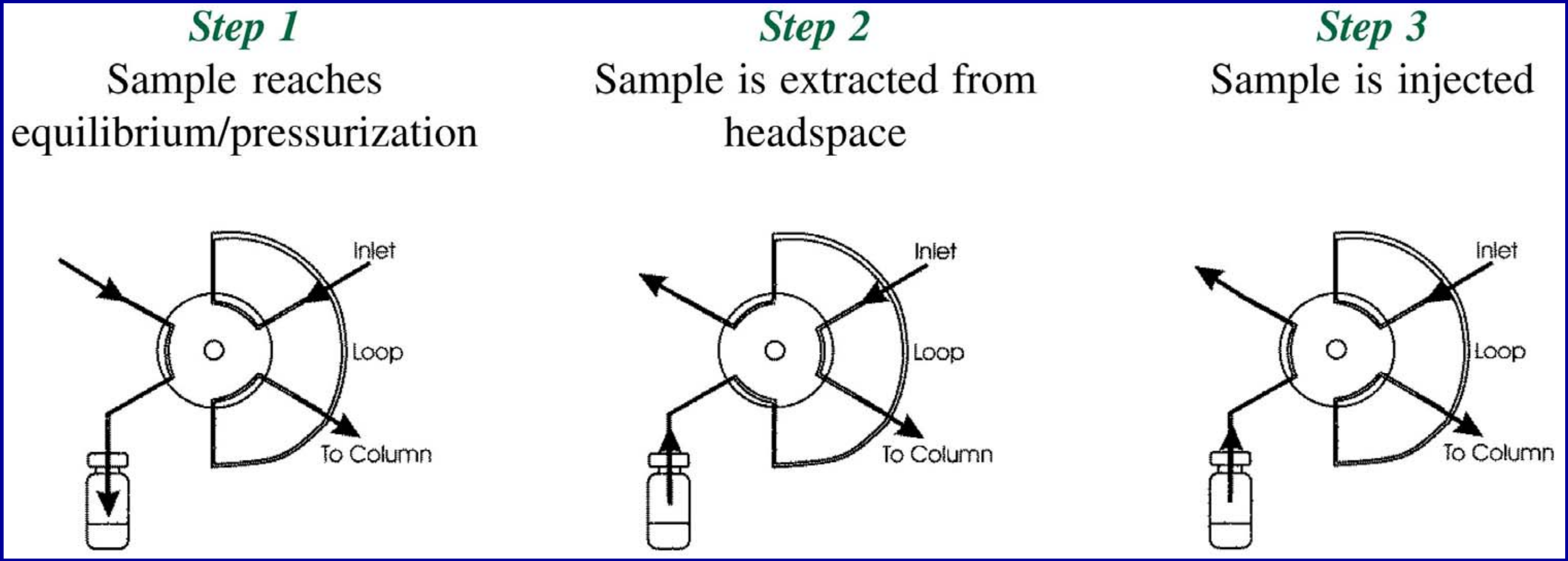
BASIC HEADSPACE CONCEPT



- A liquid or solid sample is heated in a sealed vial
- Equilibrium is established between the sample and the gas phase (headspace)
- Aliquot of the gas phase is transferred to the GC
- Not an exhaustive technique

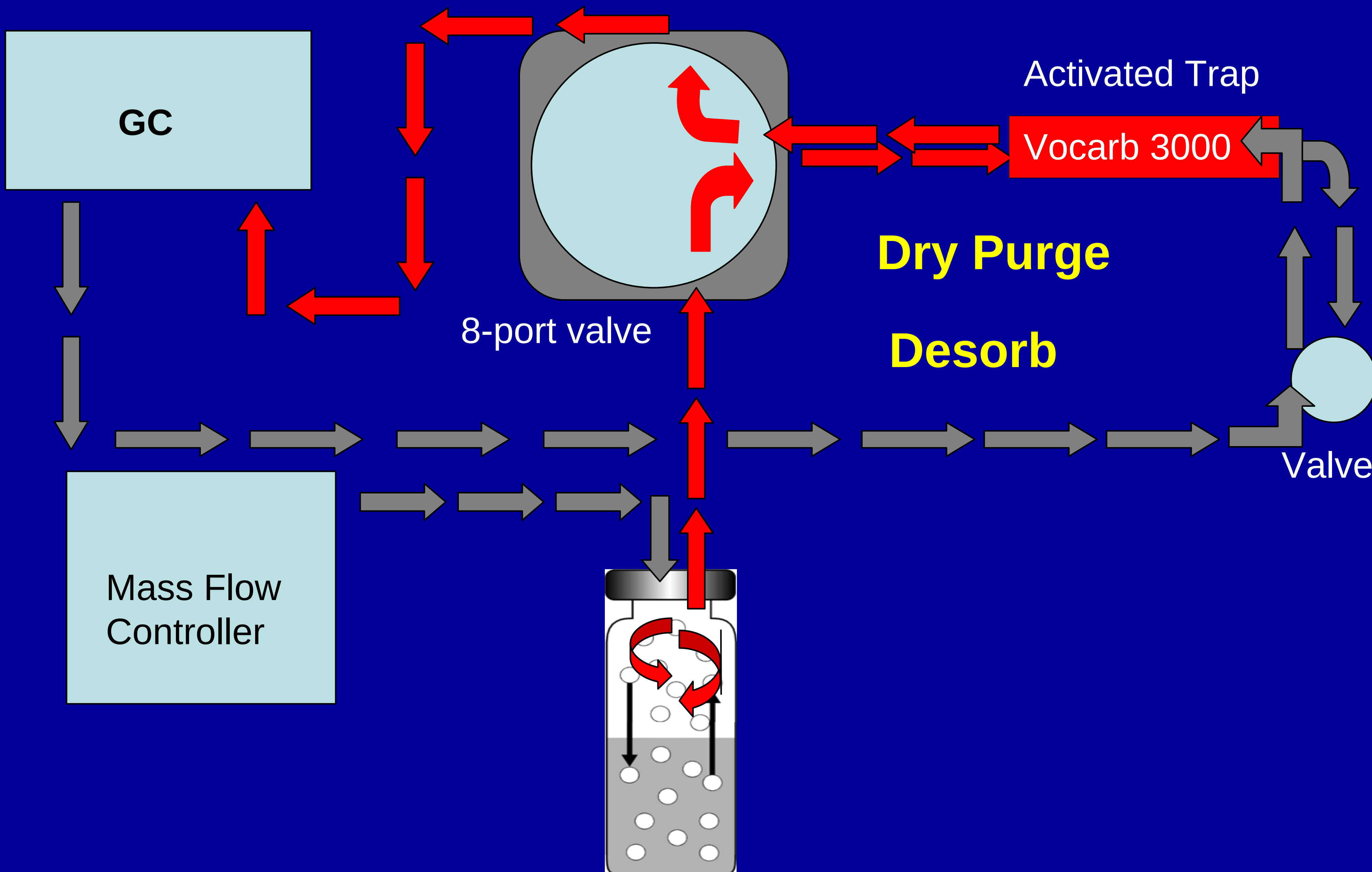
Static Headspace

Pressurized Loop System



- Only a portion of the headspace is sampled
- Not optimizing sensitivity

Dynamic Headspace



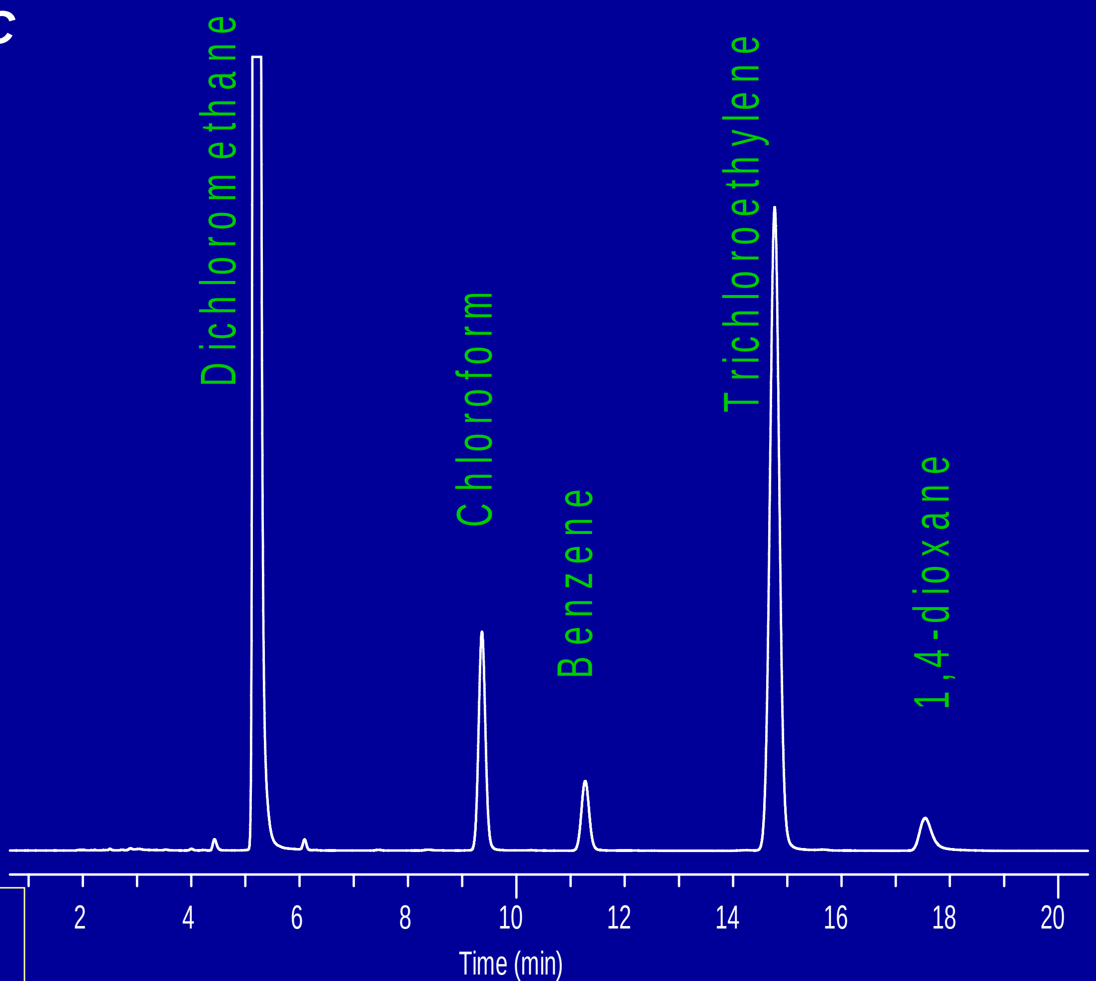
Static vs. Dynamic

Direct Sensitivity Comparison Using USP <467> Solvents

Instrument Conditions USP <467>

Sample:	***Heated for 45 minutes at 80ºC***
Injector	180ºC, split 2:1
Carrier Flow	Helium @ 5.0 ml/min, constant
Column	Rtx-G43, 30m X 0.53mm x 3.0 µm
Detector	FID 250ºC
Oven Program	40ºC for 20 minutes, to 240ºC at 25ºC/min, hold for 10 min

G43 = 6% cyanopropyl
94% dimethylpolysiloxane



Static vs. Dynamic

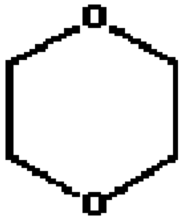
Sensitivity Comparison Using USP <467> Solvents

Analyte	Sample Conc (ppm)	Reg Limit (ppm)	Average Peak Area: Static Headspace	Average Peak Area: Dynamic Headspace	**Increase in Sensitivity**
Dichloromethane	12.0	600	619	18679	**30 X**
Chloroform	1.2	60	39	783	**20 X**
Benzene	0.04	2	15	313	**21 X**
Trichloroethylene	1.6	80	141	3479	**25 X**
1,4-dioxane	7.6	380	20	272	**13 X**

Average increase in sensitivity = 22X

Response of Polar Analyte

Why is the increase lower for 1,4-Dioxane?



Increase in sensitivity is only 13X

- Very hydrophilic compound
- Poor partitioning
- Indicates that partitioning is not the same for all compounds
- Similar for alcohols, ketones, aldehydes

Class 1 and 2 Solvents

Table I Class 1 solvents (should be avoided).

Solvent	Concentration limit (ppm)	Associated hazard
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

- **Assayed Class 2 Mix A and B on G43**
- **6ml Total Volume, 1 g Sodium Sulfate in 20 ml vial**
- **Loop at regulatory limit (1ml standard added)**
- **Trap at ½ regulatory limit (500µl standard added and increased split ratio)**

Table II Class 2 solvents.

Solvent	Concentration limit (ppm)
Acetonitrile	410
Chlorobenzene	360
Chloroform	60
Cyclohexane	3880
Dichloromethane (methylene chloride)	600
1,4-Dioxane	380
1,1,2-Trichloroethylene	80
1,2-Dimethoxyethane	100
2-Ethoxyethanol	160
2-Methoxyethanol	50
Methylbutyl ketone	50
Nitromethane	50
Sulfolane	160
Tetralin	100
Pyridine	200
Toluene	890
Formamide	220
1,2-Dichloroethene	1870
N,N-Dimethylacetamide	1090
N,N-Dimethylformamide	880
Ethylene glycol	620
Hexane	290
Methanol	3000
Methylcyclohexane	1180
N-Methylpyrrolidone	4840
Xylene	2170

Analyte List

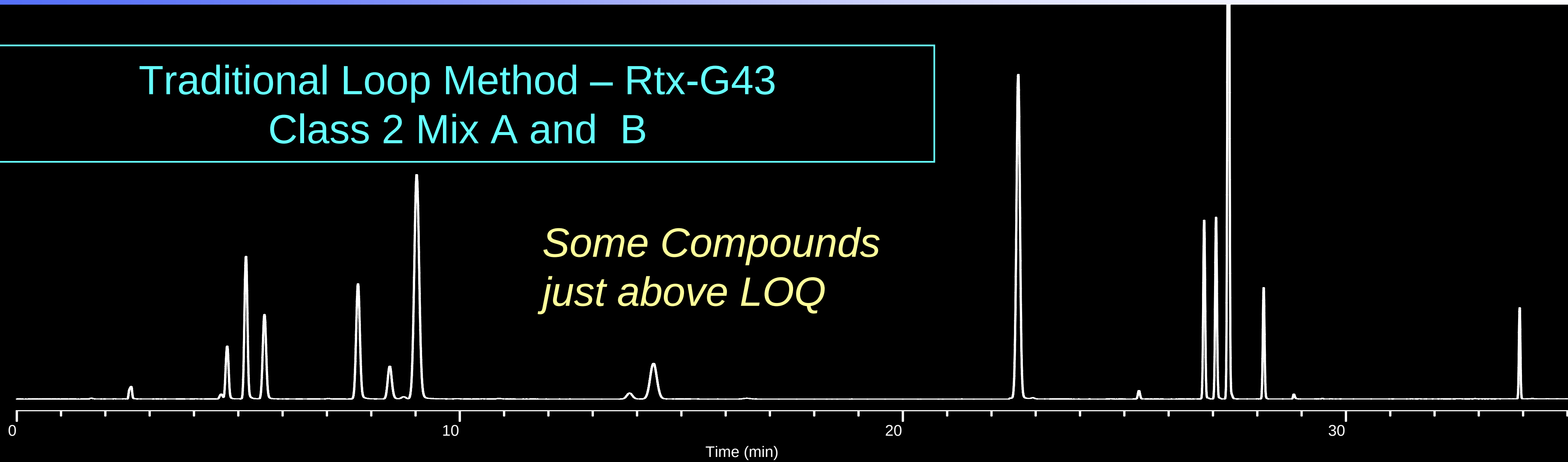
1. Methanol
2. Acetonitrile
3. Dichloromethane
4. trans-1,2-Dichloroethylene
5. Hexane
6. cis-1,2- Dichloroethylene
7. Nitromethane
8. Tetrahydrofuran
9. Chloroform
10. Cyclohexane
11. 1,2-Dimethoxyethane
12. Trichloroethylene
13. Methylcyclohexane
14. 1,4-Dioxane
15. Pyridine
16. Toluene
17. 2-Hexanone
18. Chlorobenzene
19. Ethyl benzene
20. m-Xylene
21. p-Xylene
22. o-Xylene
23. Tetralin

23 solvents with varying chemical properties

Static vs. Dynamic – A closer look

Traditional Loop Method – Rtx-G43
Class 2 Mix A and B

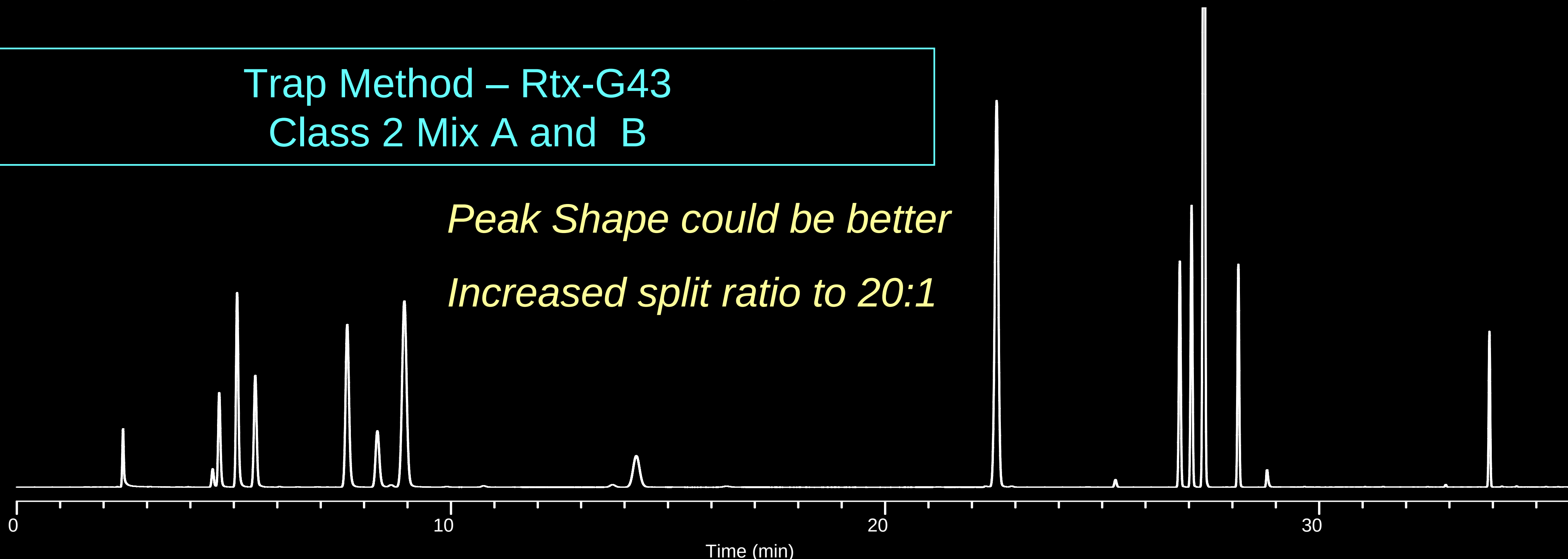
Some Compounds just above LOQ



Trap Method – Rtx-G43
Class 2 Mix A and B

Peak Shape could be better

Increased split ratio to 20:1



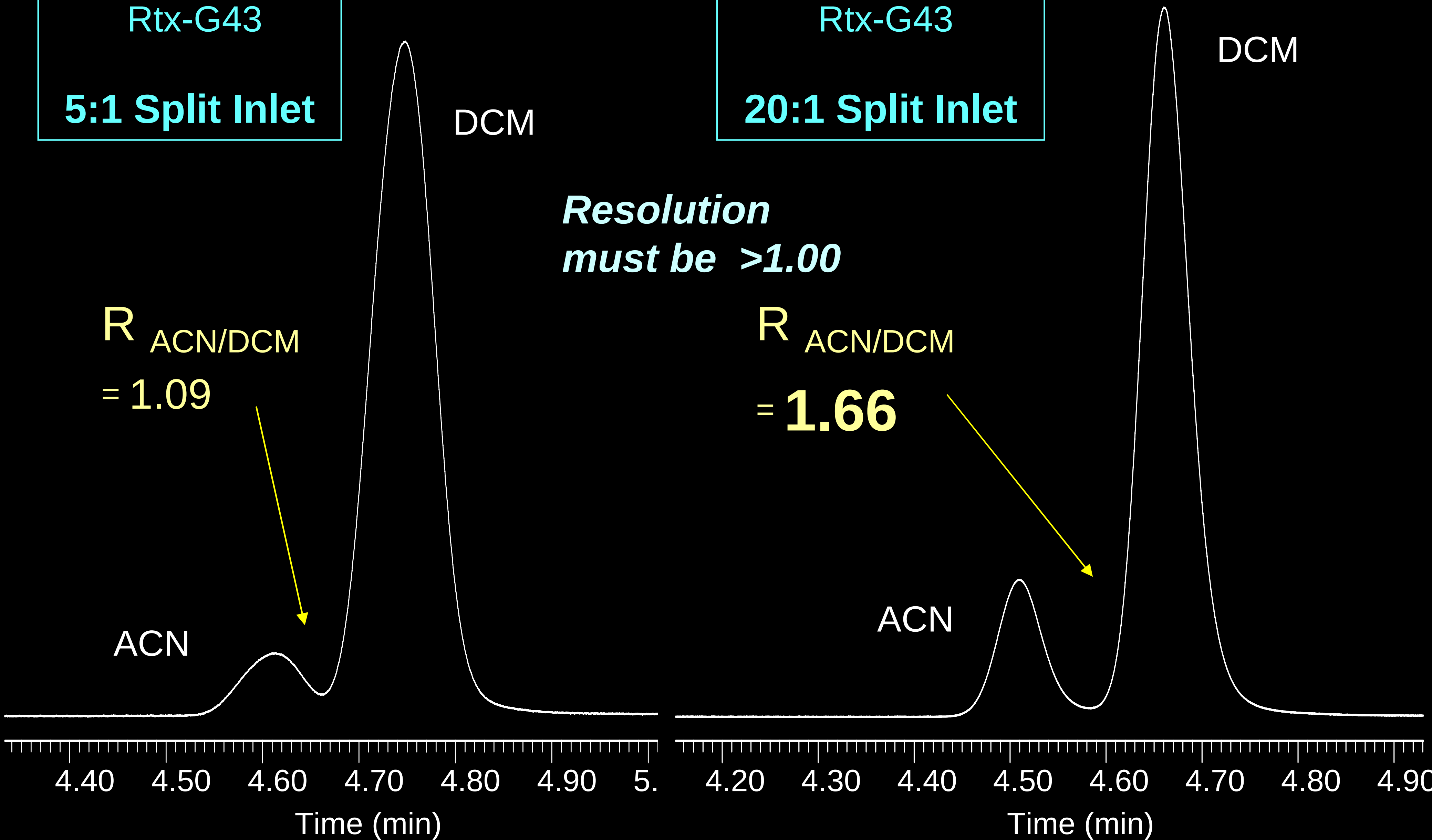
Increased Split Flow

Loop Method
Rtx-G43

5:1 Split Inlet

Trap Method
Rtx-G43

20:1 Split Inlet



Sensitivity Comparison

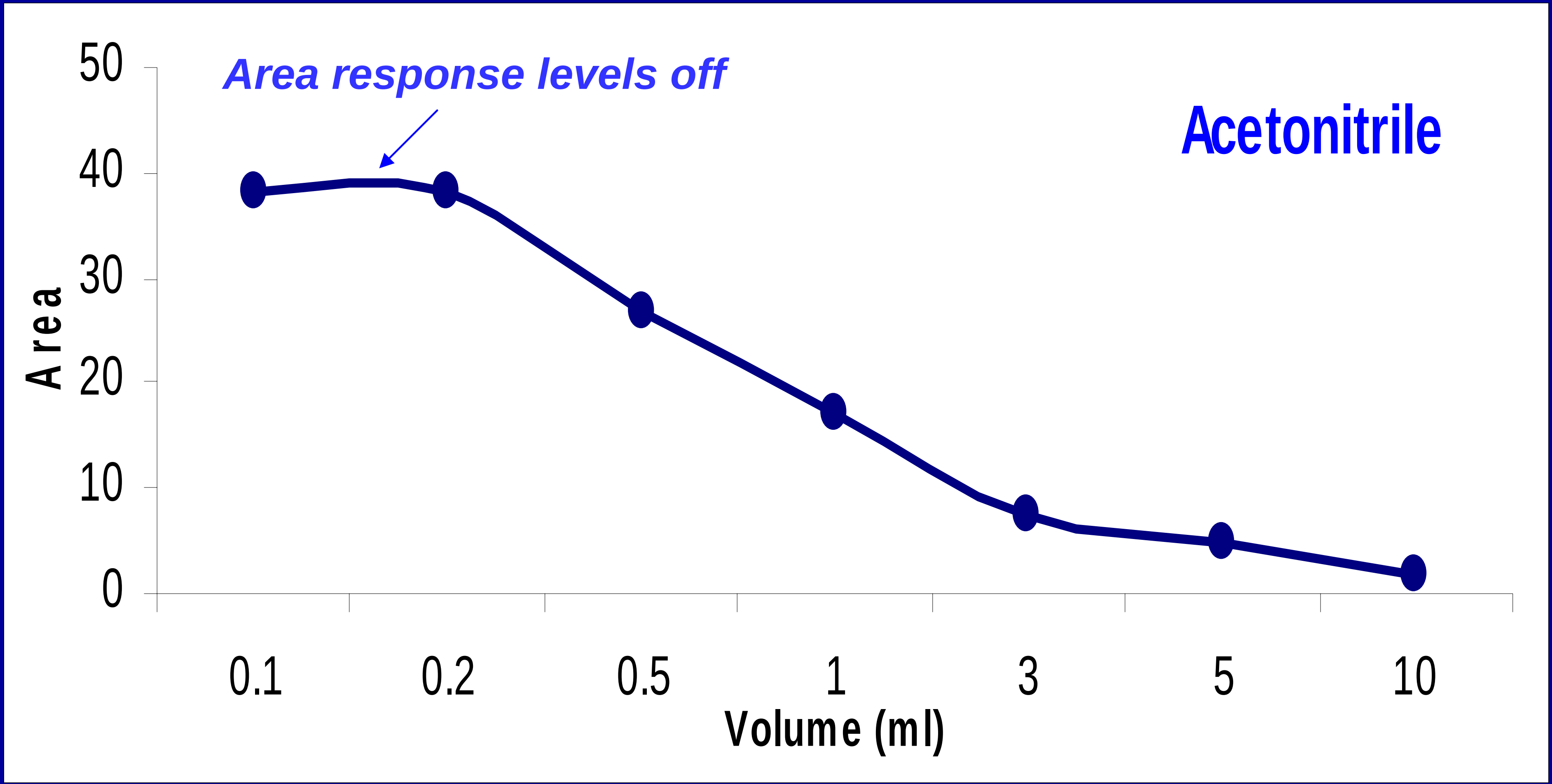
Compound	Polarity	Static Analysis		Dynamic Analysis at ½ Conc.		Increase (times)	
		Peak Area	Peak Height	Peak Area	Peak Height	Peak Area	Peak Height
Methanol	Polar	44.57097	9.68596	235.03558	83.45882	**5.3**	**8.6**
Acetonitrile	Polar	16.65052	3.79775	89.66702	26.12947	**5.4**	**6.9**
1,4-dioxane	Polar	6.83236	0.63490	14.84858	1.35838	**2.2**	**2.2**
MBK	Polar	21.91312	6.59006	35.65511	10.61567	**1.6**	**1.7**
DCM	Slightly	190.38097	41.14470	497.52942	135.51151	**2.6**	**3.3**
Hexane	Non	356.69006	65.75023	714.94470	160.13466	**2.0**	**2.5**
Calculated Average Increase of all Analytes Using ½ Conc.						**5.6**	**6.0**
			Average Increase X4 (Split)			**22**	**24**

Same average increase as noticed before

Same uneven partitioning

Effect of Phase Ratio

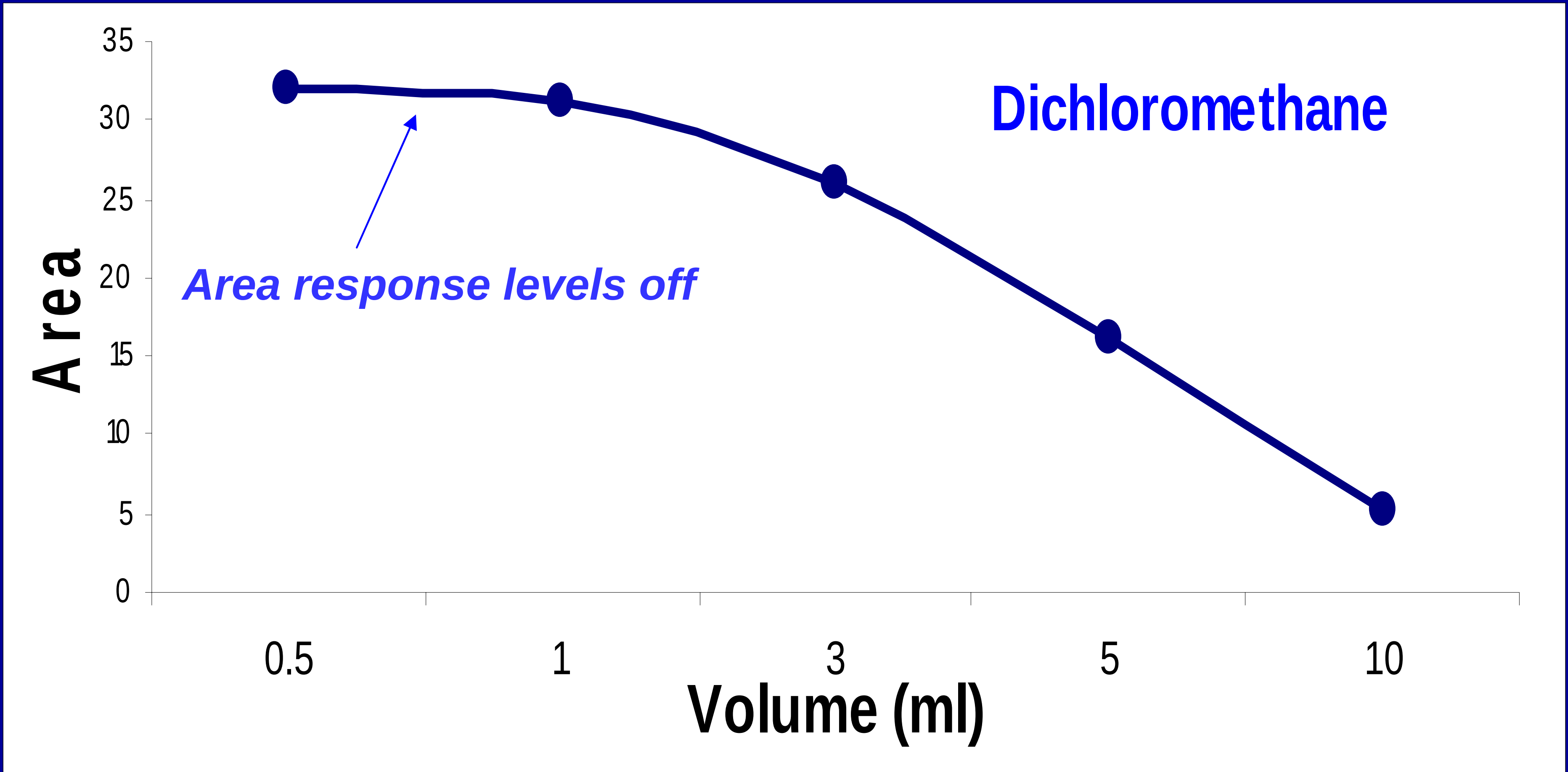
Polar Solvent (1/5 of regulatory limit)



Area of analyte increases as volume of sample decreases

Effect of Phase Ratio

Less Polar Solvent (1/5 of regulatory limit)

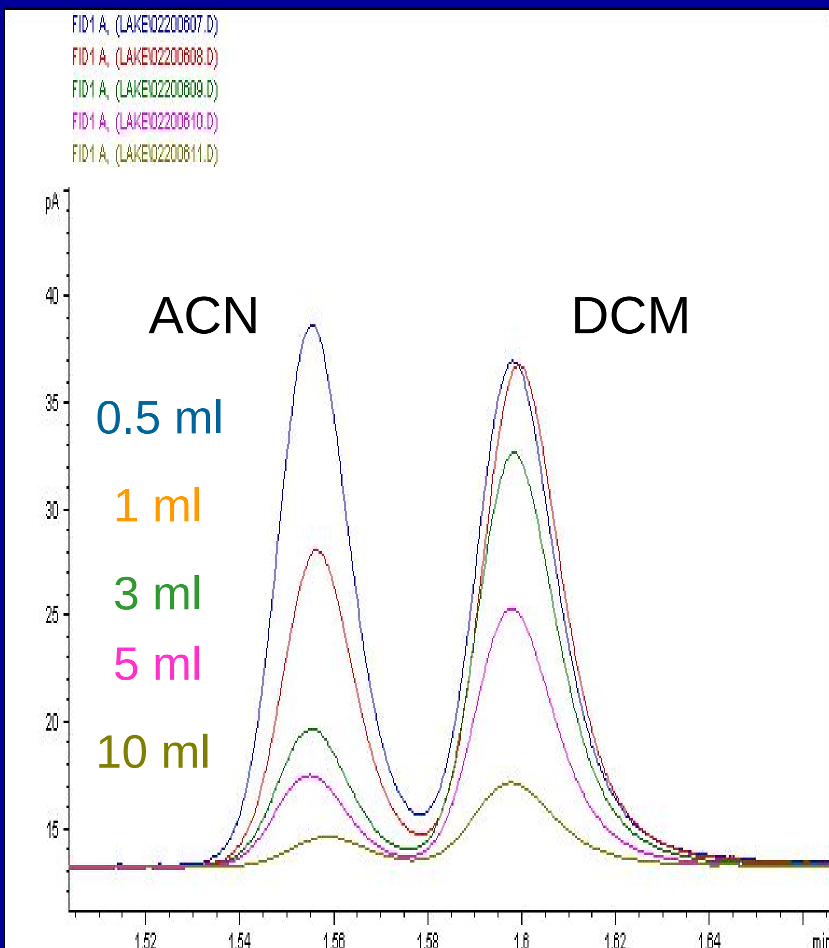


Area of analyte decreases as volume of sample increases

Effect of Phase Ratio

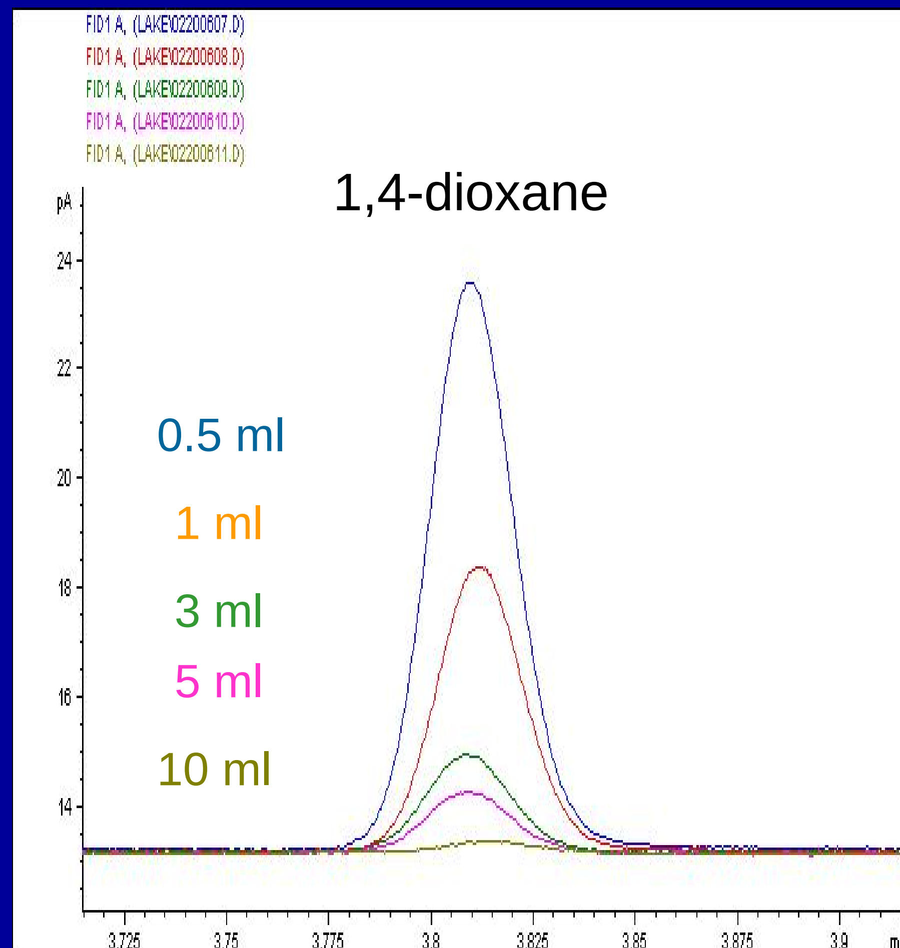
Effect of Phase Ratio on Analyte Response

Acetonitrile and Dichloromethane



10 ml to 0.5 ml range

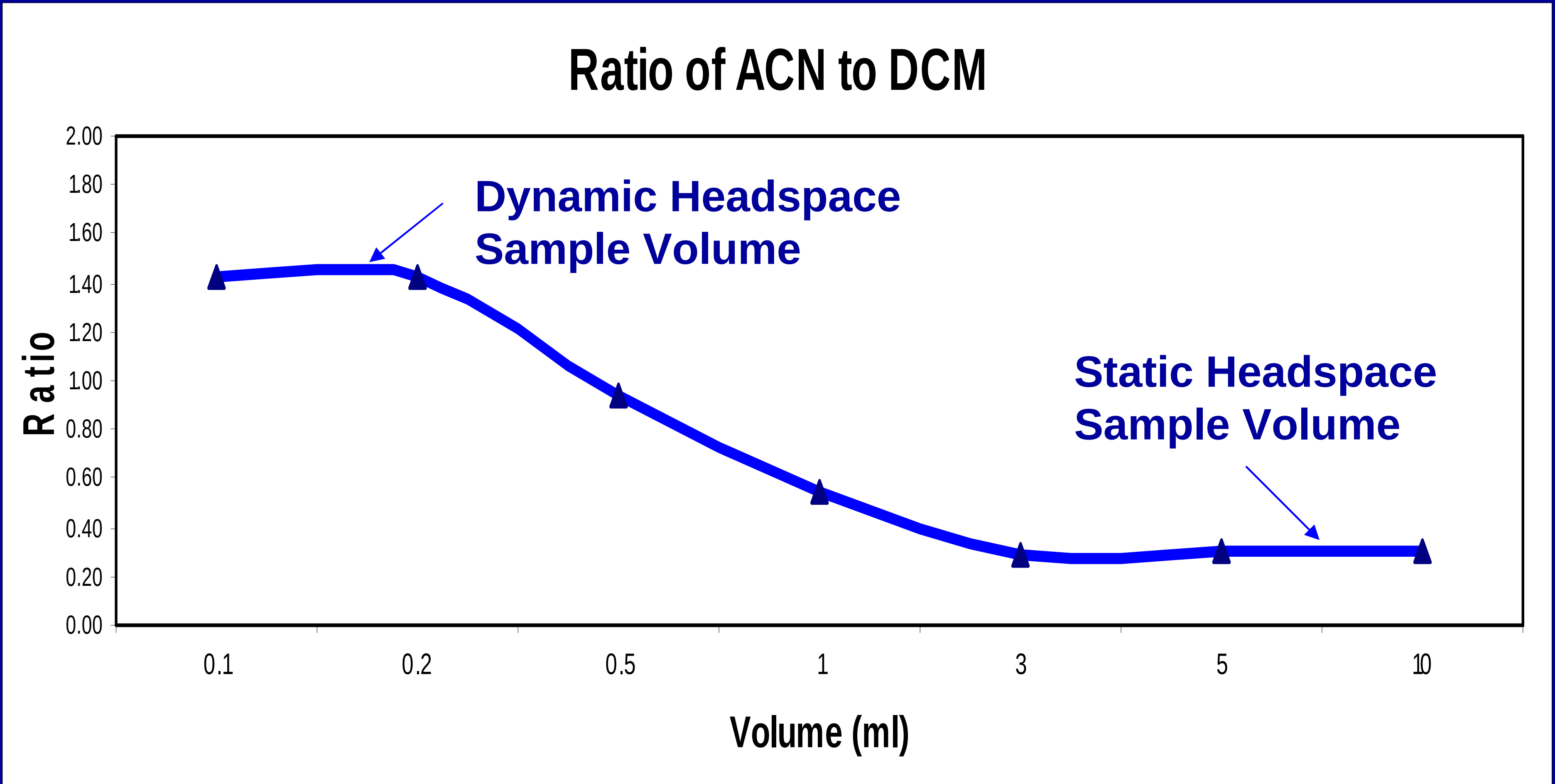
1,4-dioxane



10 ml to 0.5 ml range

Effect of Phase Ratio

Effect of Phase Ratio on Analyte Response



Smaller sample volumes are more representative of polar compounds and give better sensitivity

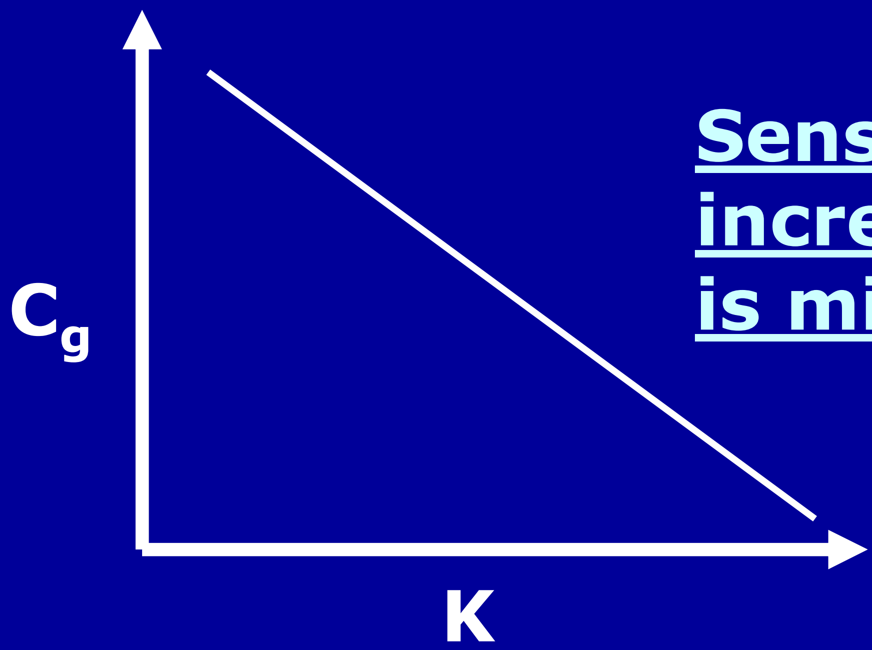
Partition Coefficient

Traditional Headspace Theory - Loop

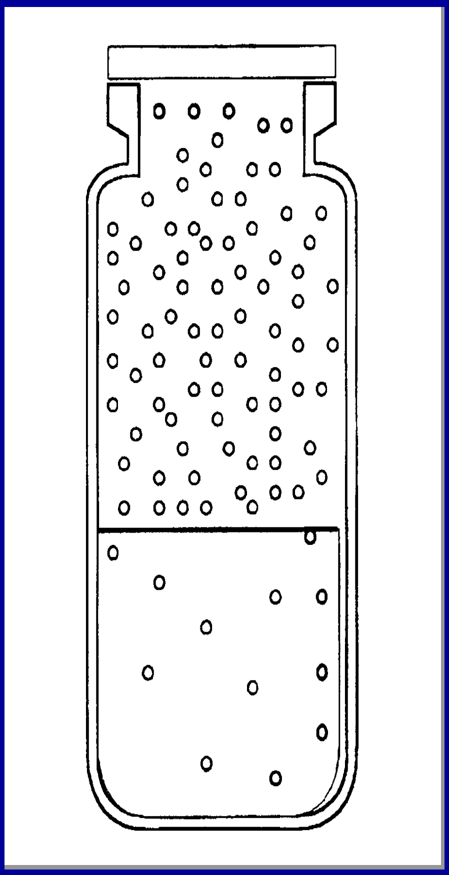
Partition Coefficient (K) = C_s/C_g

C_s = Concentration of analyte in sample phase

C_g = Concentration of analyte in the gas phase



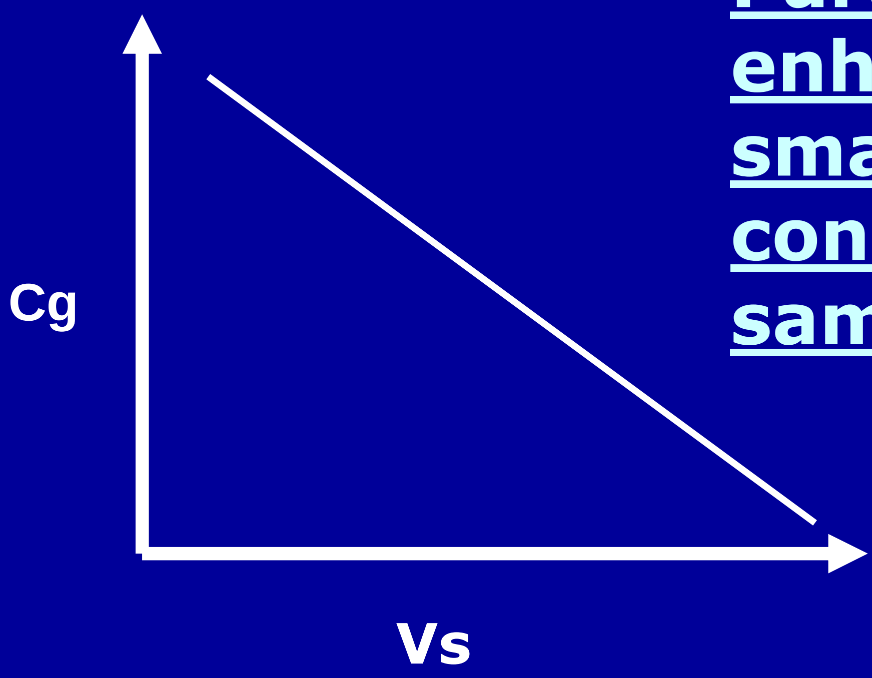
Sensitivity is increased when K is minimized.



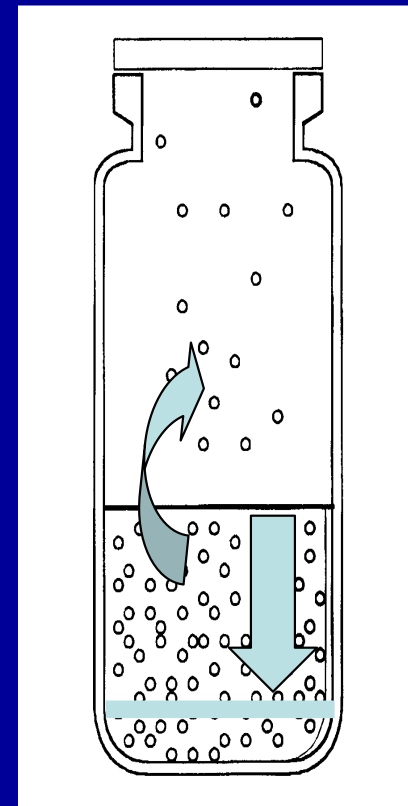
Partition Coefficient

Headspace Trap Theory

K is minimized as the volume of sample and standard decreases,



<u>Partitioning is enhanced with a smaller, less concentrated sample size</u>



80ºC- 90ºC ceiling for platen temp

Increasing Sensitivity

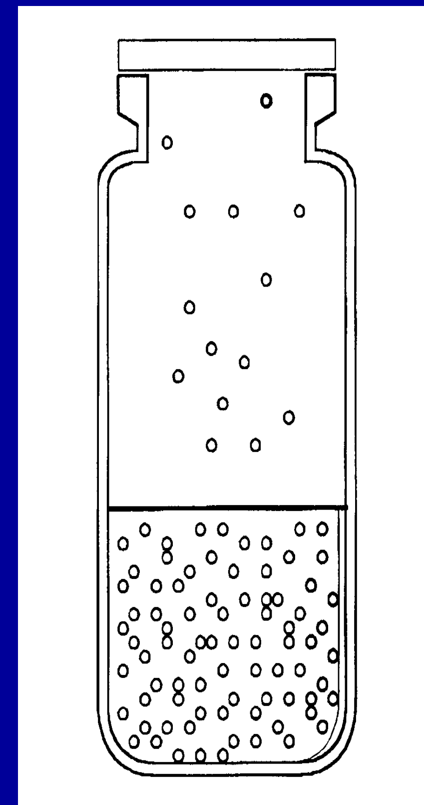
RESTEK

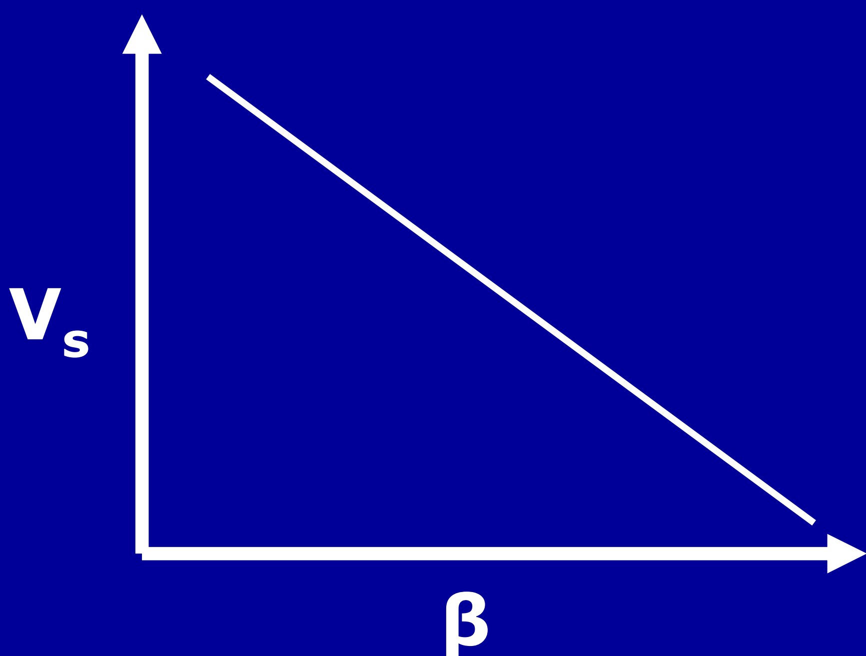
Traditional Headspace Theory - Loop

Phase Ratio (ß) = V_g/V_s

V_g = Volume of the gas phase

V_s = Volume of the sample phase



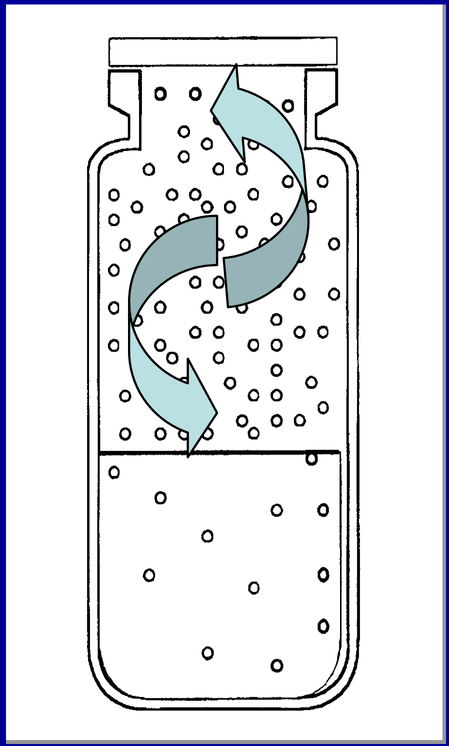


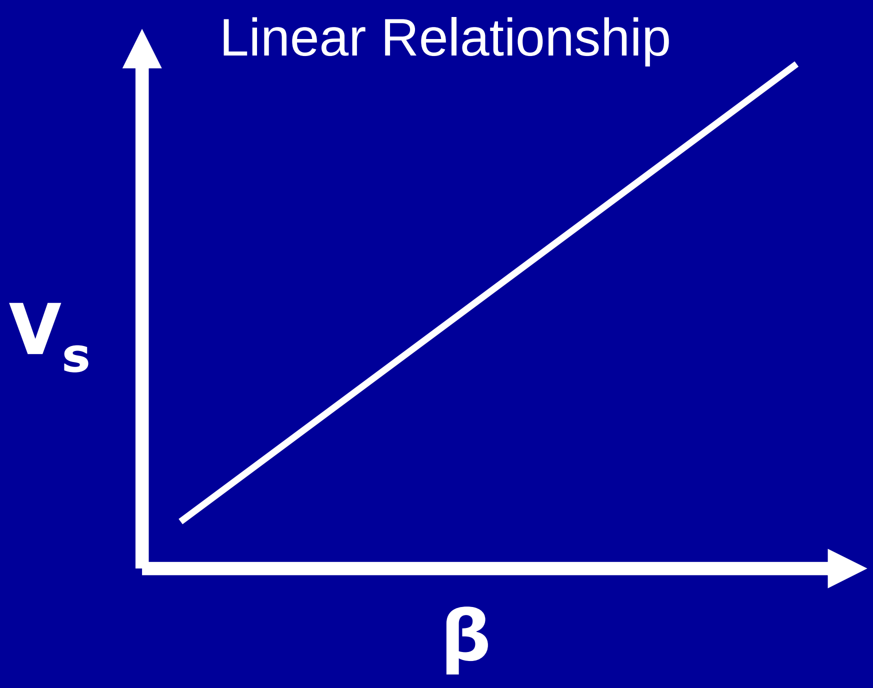
Sensitivity is increased when β is minimized.

Increasing Sensitivity

Headspace Trap Theory

Phase Ratio (ß) is now the reciprocal as an increased gas phase does not dilute sample





Sensitivity is increased when β is maximized.

Dynamic Headspace

Advantages:

1. Large Increase in Sensitivity
2. More Representative Sample
3. Smaller Volumes = Smaller Sample Sizes
   = Faster sample
   equilibrium times

Makes Possible:

1. More Versatility in Instrument Conditions
2. Dual Column FID Method for Analyte Confirmation – *using two alternatively selective columns*

Headspace Conditions

Dynamic Headspace (Trap) Conditions	
Instrument	Teledyne Tekmar HT3
Valve Oven Temp	220ºC
Transfer Line Temp	220ºC
Standby Flow Rate	50 ml/min
Trap Standby Temp	40 ºC
Platen/Sample Temp	80 ºC
Sample Equil. Time	15.00 min
Mixer Time	2.00 min
Mixing Level	5
Mixer Stabilize Time	0.50 min

Sweep Flow Rate	75 ml/min
Sweep Flow Time	5.00 min
Dry Purge Time	10.00 min
Dry Purge Flow	100 ml/min
Dry Purge Temp	25ºC
Desorb Preheat	245 ºC
Desorb Temp	250ºC
Desorb Time	1.00 min
Trap Bake Temp (K)	260ºC
Trap Bake Time	6.00 min
Trap Bake Flow	450 ml/min

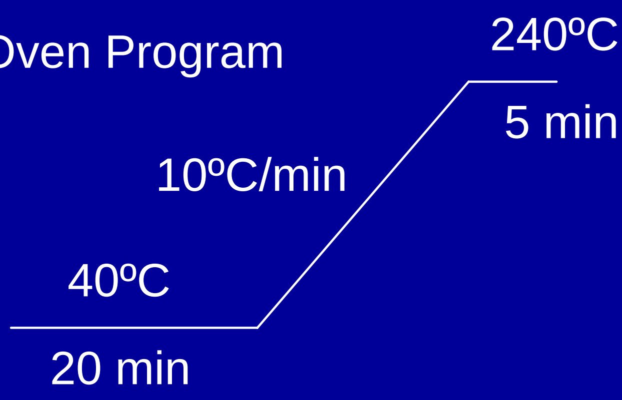
Dual Column Assay

Instrument	Agilent 6890 GC/FID
Injector, Split Mode	220ºC
Split Ratio	20:1
Column Flow	Helium, constant flow, split into two columns A: 1.5 ml/min B: 1.6 ml/min
Column	A: Rtx-G43, 30m X 0.32mm x 1.8 µm B: Stabilwax, 30m X 0.32mm x 1.0 µm
Detector, FID	250ºC
Hydrogen	40 ml/min
Air	450 ml/min
Make-up	45 ml/min

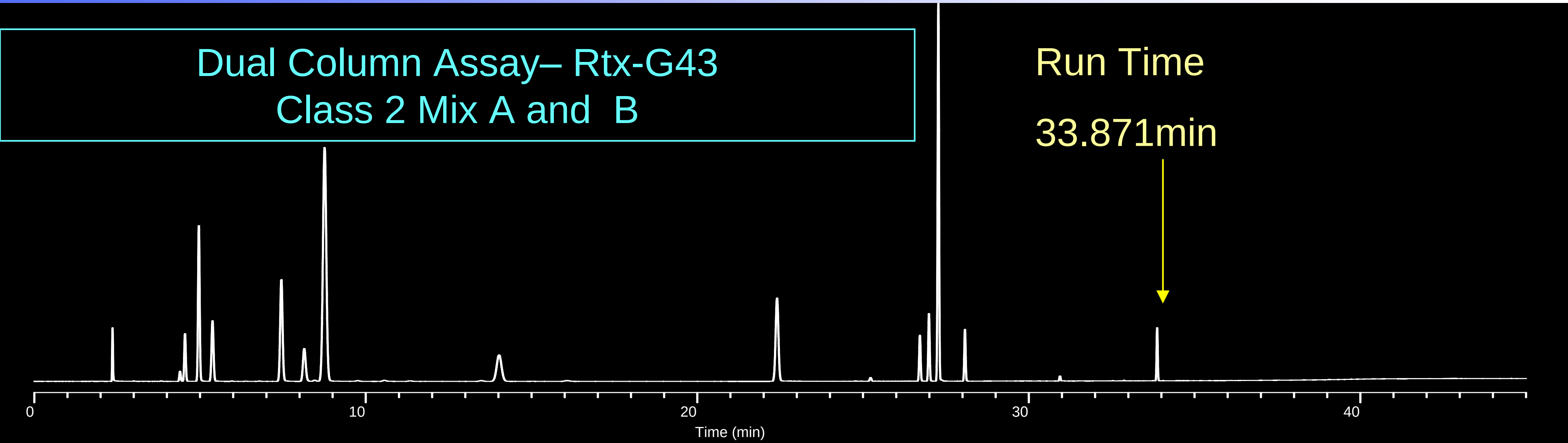
G43 = 6% cyanopropyl 94% dimethylpolysiloxane

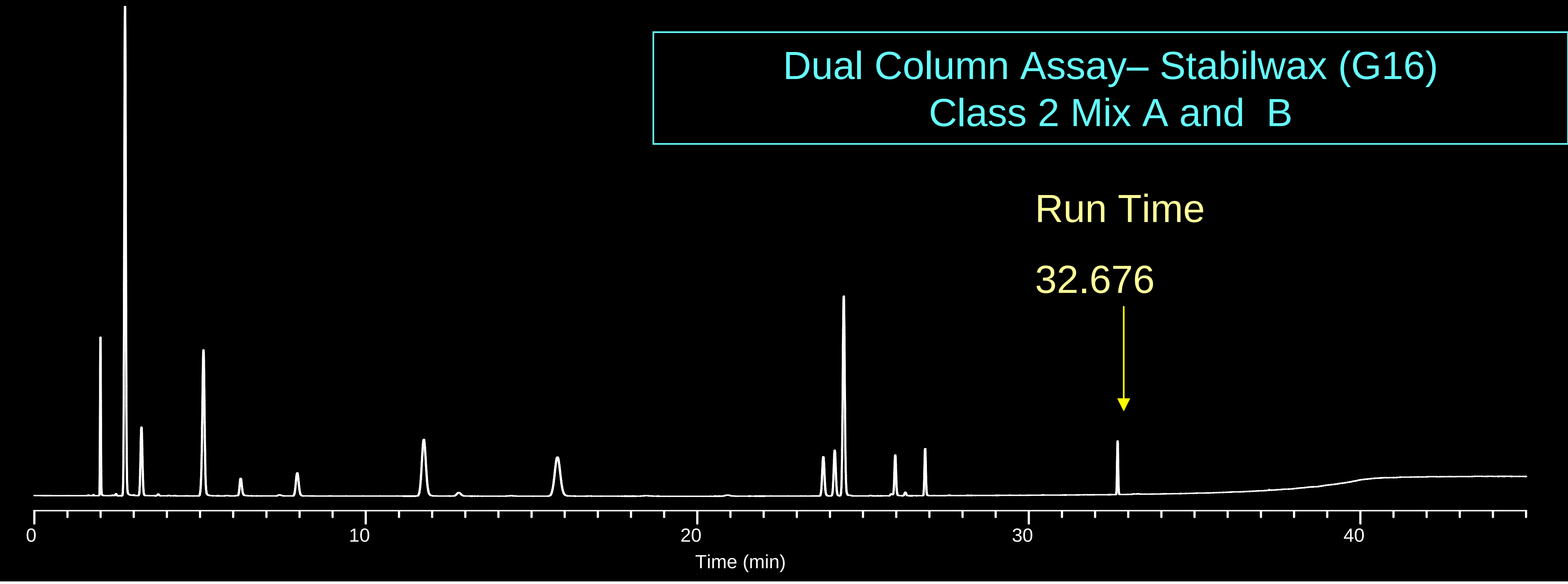
G16= 100% Polyethylene glycol (PEG)

Oven Program



Dual Column Assay





Dual Column Assay

- Coelutions:

G43

1. Cis-1,2-dichloroethane / Nitromethane
2. Pyridine / Toluene
3. m-xylene / p-xylene (critical)

G16

1. Tetrahydrofuran / Trans-1,2-dichloroethane
2. Cis-1,2-dichloroethane / Trichloroethylene (critical)

- One oven hinders selectivity
- Lengthy run times

Instrument Configuration

Instrument Configuration

Comprehensive Method

RESTEK

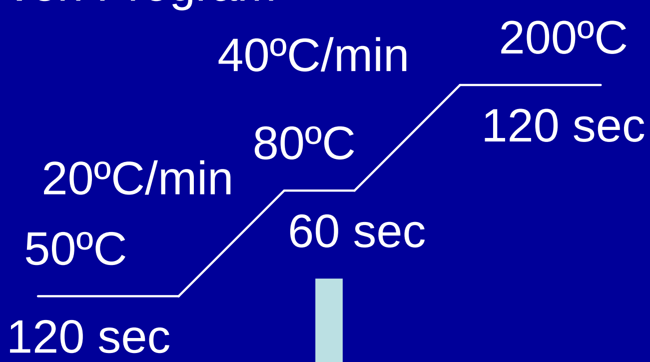
Headspace Method – same as previous

0.5 ml and 1 ml total volume
in 20ml headspace vial

Instrument	Agilent 6890 GC/FID/FID
Injector, Split Mode	220ºCm split ratio 20:1
Column Flow	Helium, constant flow, split into two columns (0.32mm guard)
Oven Program	220ºC

Column A (G43) – Rtx-624
20m x 0.18mm x 1.0µm
Flow: 0.85ml/min

Oven Program

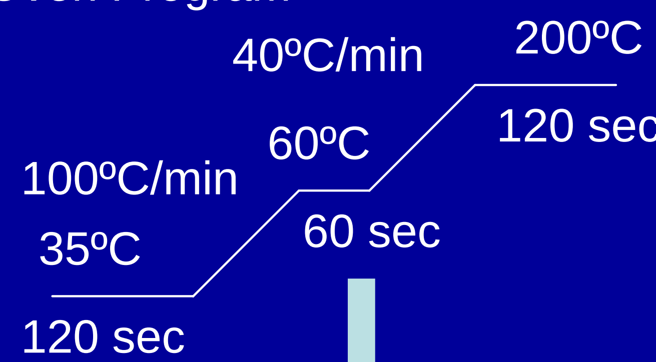


FID A - 250ºC

Hydrogen	40ml/min
Air	450 ml/min
Makeup (He)	45 ml/min

Column B (G16) – Rtx-WAX
20m x 0.18mm x 0.4µm
Flow: 0.99ml/min

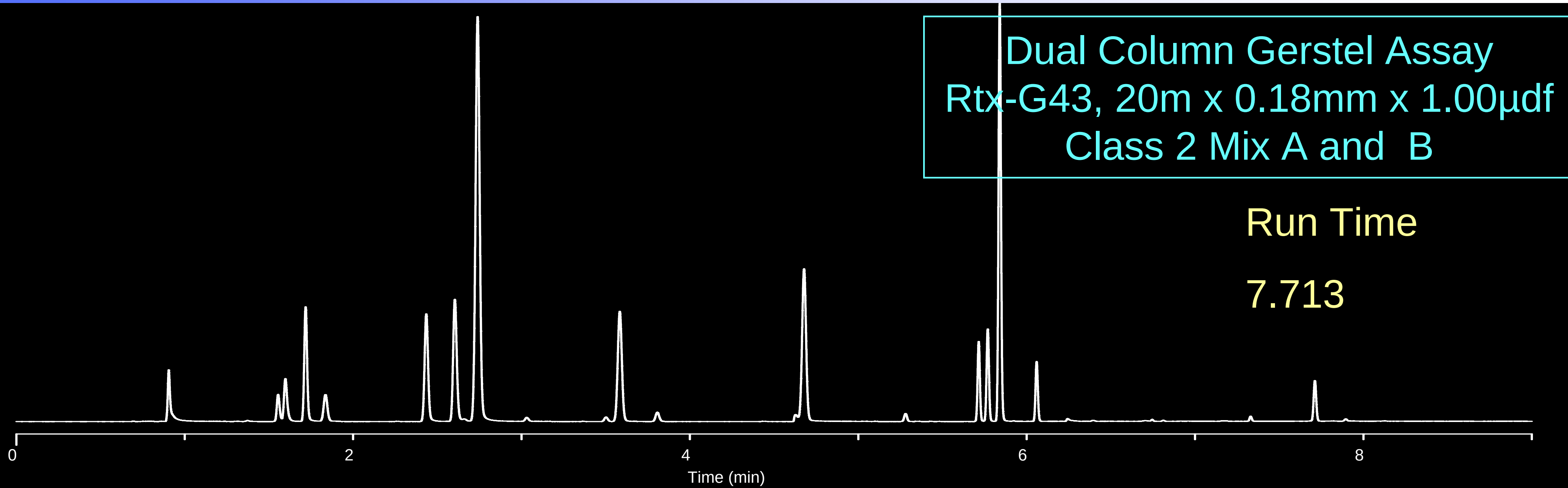
Oven Program

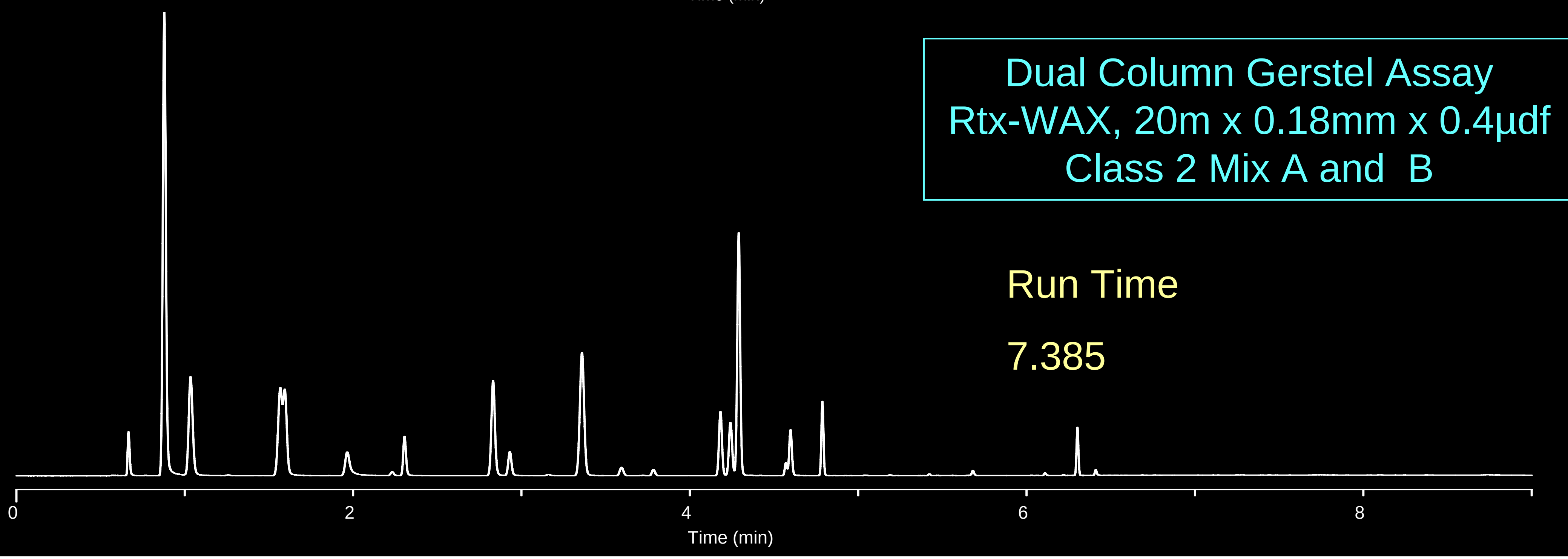


FID B - 250ºC

Hydrogen	40ml/min
Air	450 ml/min
Makeup (He)	45 ml/min

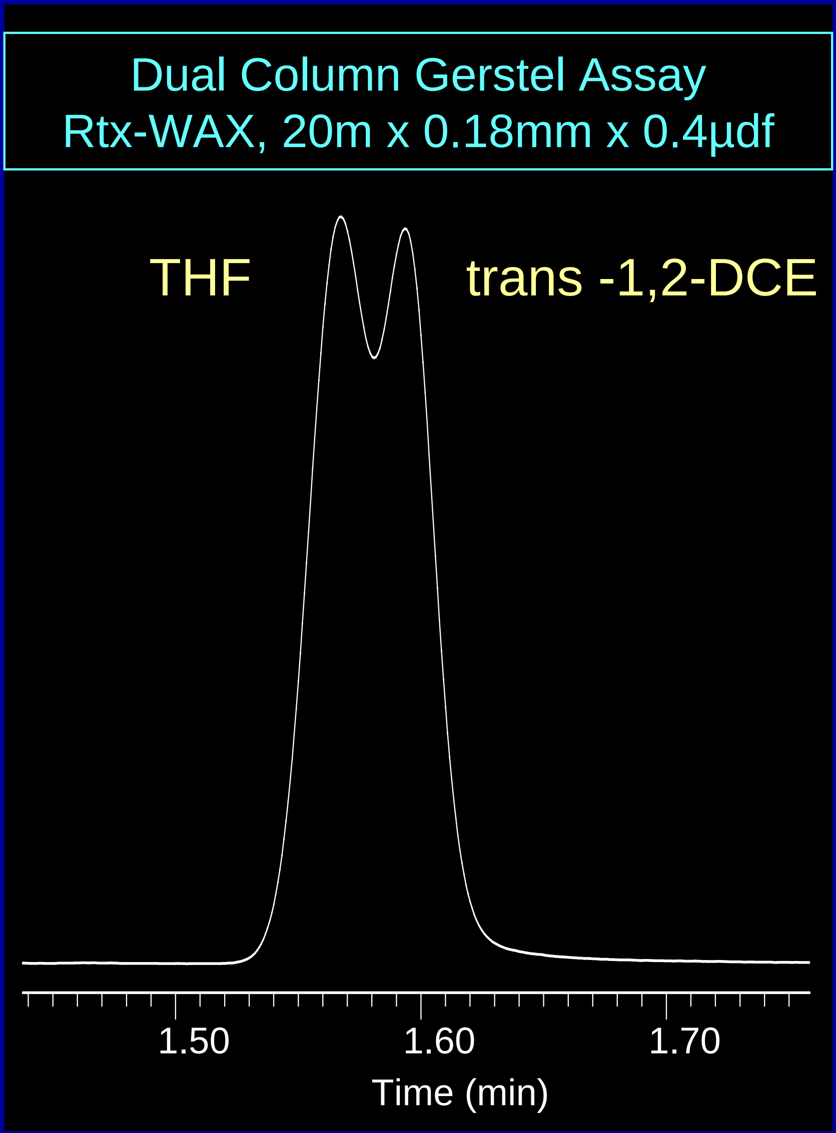
Method Results

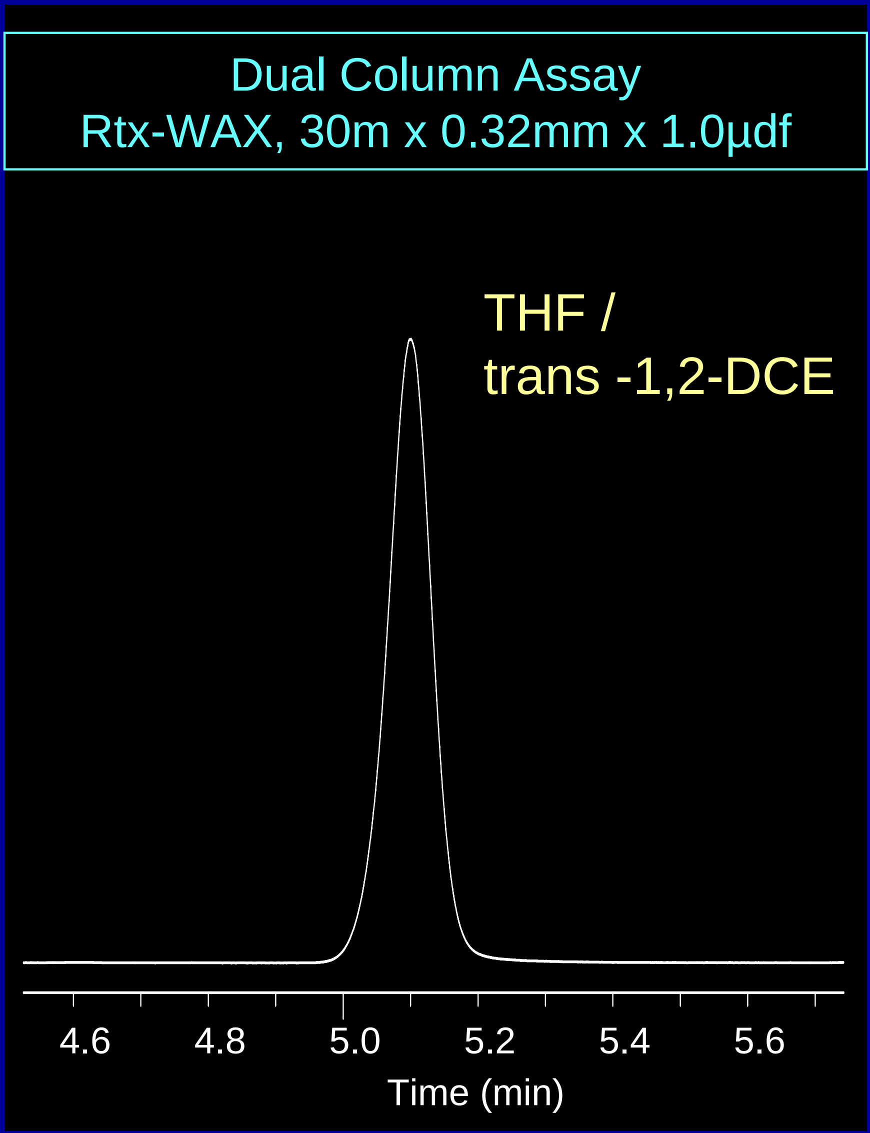




Method Selectivity

Equal or Enhanced Selectivity – G16



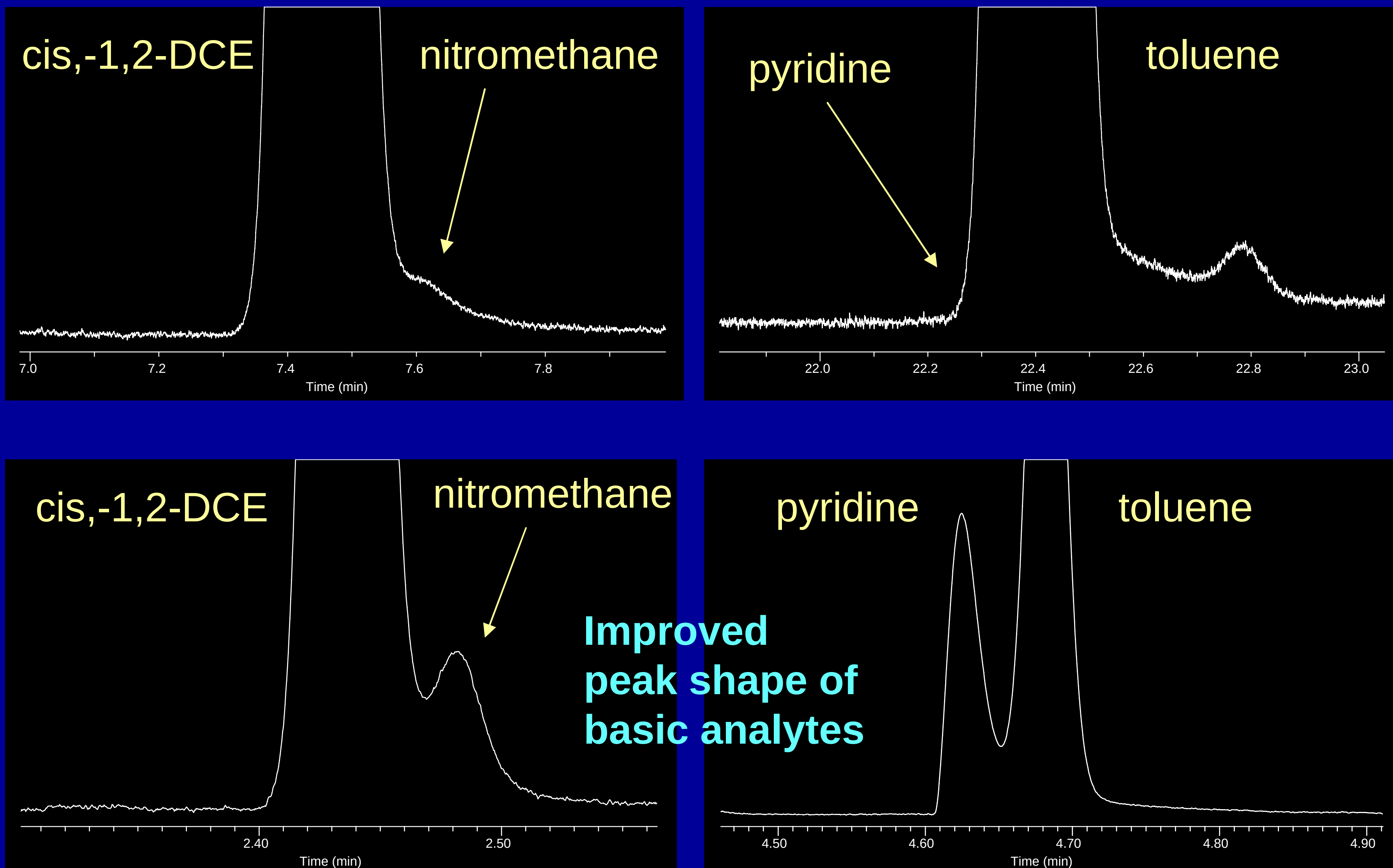


Method Selectivity

Equal or Enhanced Selectivity – G43

Dual Column
30m x 0.32mm x 1.0µdf

Dual Column Gerstel
20m x 0.18mm x 0.4µdf



Method Sensitivity

Sensitivity Comparison Using Peak Height

Method	Sample Volume	Average Increase of all Analytes
Standard Loop	6 ml	---
Standard Trap	6 ml	**24 X**
Gerstel Dual Column Assay	1 ml	**313 X**

Cumulative effect of trapping, sample volume, and peak height increase using microbore columns

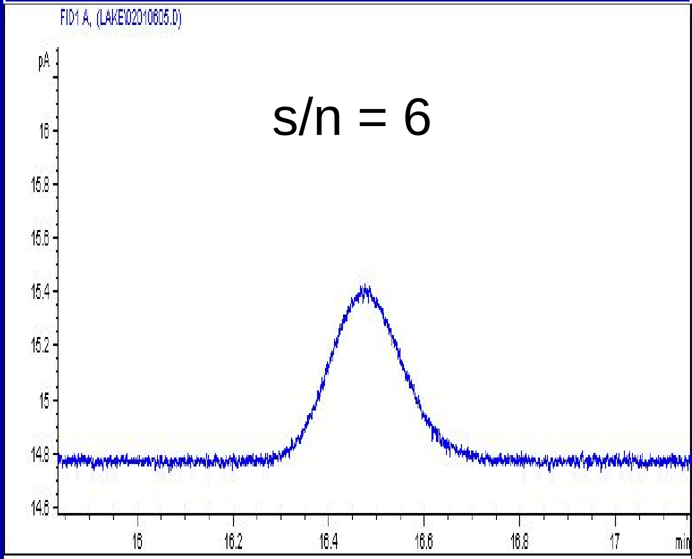
Effect of Polarity on Sensitivity

Solvent	Polarity	Increase
Methanol	Polar	711 X
Acetonitrile	Polar	801X
MBK	Polar	79X
DCM	Slightly Polar	74X
Hexane	Non Polar	29X

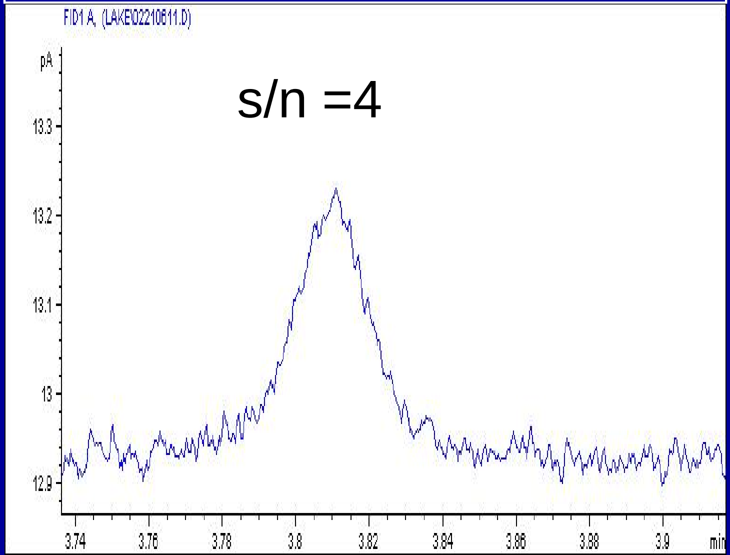
Method LOD

Sensitivity of 1,4-dioxane as a function of signal–to-noise

Traditional Loop Method
19 µg in 6ml volume
20 ml headspace vial



Trap-Mach Method (split)
19 ng in 0.5ml volume
20 ml headspace vial



All factors considered, approximately a 700 fold increase in signal response for polar compounds, even when column flow is split

Method Linearity and Range

1 ml Sample Volume

Linearity –in 20ml headspace vial

Correlation Coefficient >0.994 for all analytes

Average Correlation Coefficient = 0.9984

Range – various low responders - 1,4-dioxane = 0.19µg to 1.9µg

- MBK = 0.03µg to 0.3µg

0.5 ml Sample Volume

Linearity –in 20ml headspace vial

Correlation Coefficient >0.995 for all analytes

Average Correlation Coefficient = 0.9982

Range – various low responders - 1,4-dioxane = 0.019µg to 0.76µg

- MBK = 0.003µg to 0.12µg

Method Reproducibility

Acetonitrile Reproducibility

40µl of standard / 0.5ml sample
1.64µg/ml or 4% of reg limit
@ reg limit using 10mg sample

Rep	Solvent	Peak Area	Peak Height	RT (min)
1	ACN	37.8979	32.92955	1.556
2	ACN	35.9404	31.11098	1.556
3	ACN	37.76856	32.61021	1.554
4	ACN	37.16529	31.81859	1.555
5	ACN	35.05855	30.41616	1.554
6	ACN	38.60516	33.66129	1.553
	Avg	37.0726	32.0911	1.555
	Std Dev	1.331	1.207	0.001
	%RSD	3.591	3.760	0.078

Dichloromethane Reproducibility

40µl of standard / 0.5ml sample
2.40µg/ml or 4% of reg limit
@ reg limit using 10mg sample

Rep	Solvent	Peak Area	Peak Height	RT (min)
1	DCM	40.34903	28.88841	1.599
2	DCM	41.48586	30.95327	1.599
3	DCM	40.88852	30.27963	1.557
4	DCM	41.47880	30.21242	1.557
5	DCM	41.25531	30.57424	1.557
6	DCM	41.12213	30.62504	1.556
	Avg	41.0966	30.422	1.598
	Std Dev	0.430	0.373	0.001
	%RSD	1.047	1.226	0.077

Method Time

USP method:

2 sample preparations at 60 minutes each

= 2 hours/sample

Headpspace Trap with MACH:

1 sample preparations = 33 minutes /sample

(Limited by headspace analysis)

Approximate reduction of 1.5 hours / sample

Conclusion

- Better sensitivity - sample sizes of 25mg to 10mg possible
- Selectivity and Confirmation -Choosing two columns with alternate selectivity gives analyte confirmation for all ICH compounds
- More representative analysis of polar compounds
- Shorter analysis and sample equilibration times - Higher throughput

Thank You