Avantor® Hichrom GC Method Development

Kirsty Ford

Senior Chromatography Technical Specialist

In Partnership with MAC-MOD, our authorized channel partner. MAC-MOD have been a trusted partner for over 20 years with expertise in HPLC & UHPLC

Agenda

01

Establish objectives, tools and selectivity

02

Goals of optimizing GC parameters

03

Standard GC method development, split and splitless

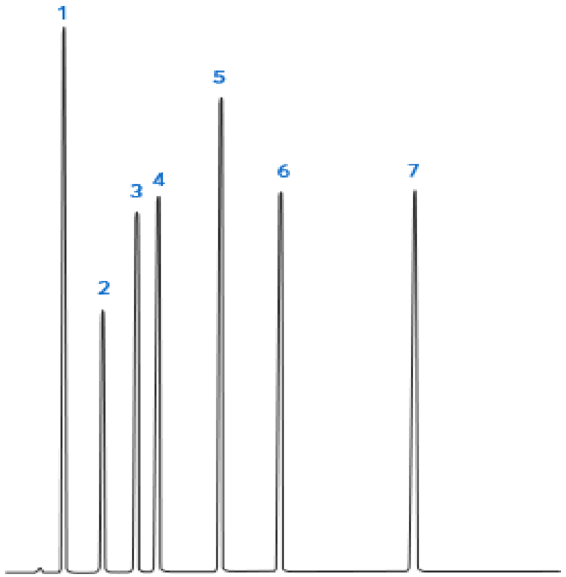
04

Fast GC with method transfer guidance

Where to start with GC method development

Establish objectives, tools and selectivity

- Analytes of interest
- Aim of analysis/Application
- GC configuration
- GC column phase
- Sample mixture



Separation of organophosphate pesticides using the HI-1701-
https://av.cmd2.vwr.com/pub/apl/chrom/main?key=C-12999

avantor™

1. Analytes of interest

What are the analytes of interest?

Are they suitable for GC?

What physiochemical properties will influence the GC parameters?

- Analytes of interest

Analytes of interest suitable for GC?

Volatiles, semi volatiles & permanent gases = GC

- Low BP (<400 °C)
- Easily vaporised/Volatile
- Low molecular weight (Approx 800 Da)
- Stable at high temp.
- High vapour pressure
- Organic compounds

Non volatiles & volatiles = HPLC

- Usually higher BP, or decomposes before BP
- Soluble in a liquid phase
- Low to high molecular weight (<500,000 Da)
- Denatures at higher temp/stable at lower
- Contains salts, can carry a charge
- Organic and Inorganic compounds

Example - Essential oils

Which is more suitable, GC or HPLC?

Analytes of interest suitable for GC?

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- Easily vaporised/Volatile
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- Soluble in a liquid phase
- Low to high molecular weight (<500,000 Da)
- Denatures at higher temp/stable at lower
- Contains salts, can carry a charge
- Organic and Inorganic compounds

Essential oils

- Low BP, approx 100-200°C
- Easily vaporised/Volatile
- MW usually <500 Da
- Thermally stable, decompose >400°C
- High vapour pressure
- Organic compounds
- Soluble in liquid phase

1 Analytes of interest – Physiochemical properties

The properties influence method direction

Polarity – Column phase selection.

Boiling point – Inlet temperature and oven temperature.

Similar or different boiling points of analytes – Column phase selection.

Structural isomers – If separation wanted, mid to high polarity phase required.

Non labile or labile compounds - Labile compounds "Softer" conditions needed, lower initial temperatures.

Sample matrix, clean or dirty – Column dimensions, liner choice, sample prep process.

2. Aim of analysis/Application

What is the analysis to achieve?

What application notes suit the aim of analysis?

Are there application specific columns available?

- Analytes of interest
- Aim of analysis/Application

2. Aim of analysis/Application

What information is required from the results?

- New method or established method?
- Is the analysis of a simple or complex sample?
- Do all analytes need to be detected and separated?
- High level or low-level resolution required?
- Are application notes available?
- What level of sensitivity is required?

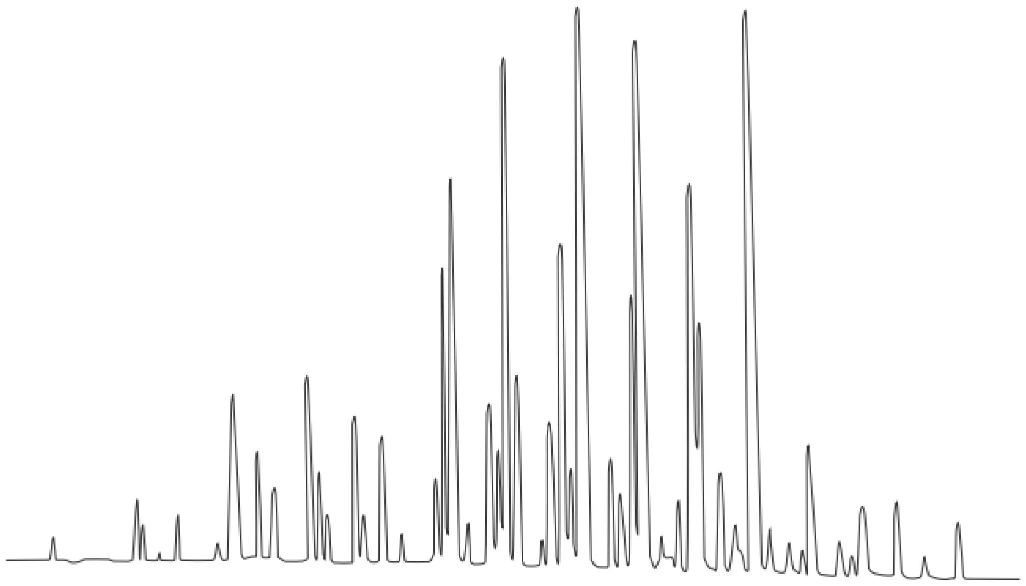

Figure 1: Quick screening of the Aroclor 1254 PCB mixture using the Avantor® Hichrom HI-SE54 phase.

https://av.cmd2.vwr.com/pub/apl/chrom/main?key=C-13114

Drives decisions on products and GC configuration suitability

2. Aim of analysis/Application

What information is required from the results?

- New method or established method?
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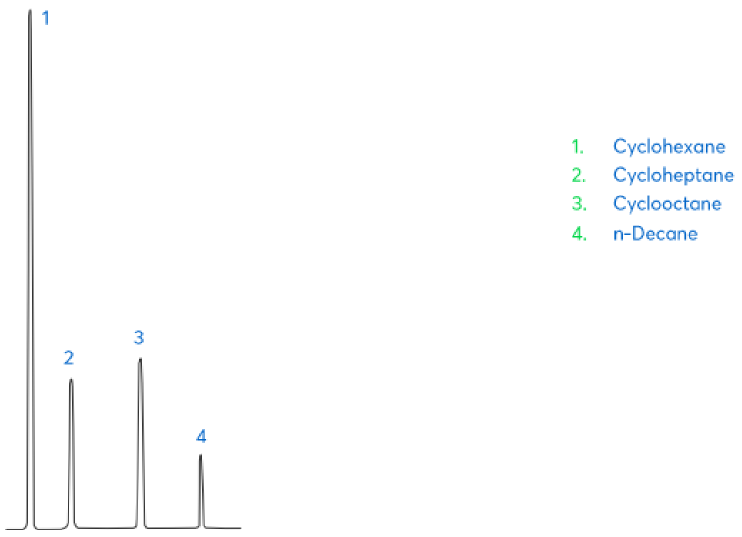


Figure 1: Analysis of cyclic hydrocarbons using the Avantor® Hichrom HI-1 phase.

https://av.cmd2.vwr.com/pub/apl/chrom/main?key=C-13098

Drives decisions on products and GC configuration suitability

2. Aim of analysis/Application – App. Specific columns

Check available resources -

1. Avantor GC phase document and cross reference document.

Phase	Functional group	Max. Temp.*	Crossbond	Application areas	Methods
APOLAR					
HI-1	100% Methyl Polysiloxane (100% Dimethylpolysiloxane)	350 °C	Yes	General purpose apolar phase - Solvent impurities, PCBs, Simulated Distillation, drugs, natural gases, hydrocarbons, essential oils, semivolatiles, pesticides, phenols	EPA: 504.1, 505, 551, 606, 612, 8141A/B USP: G1, G2, G9, G38
HI-1 HT	100% Methyl Polysiloxane (100% Dimethylpolysiloxane) - High Temperature	400 °C	Yes	High Molecular Weight Waxes, Motor Oils, Polymers/Plastics, Simulated Distillation	USP: G1, G2, G9, G38
HI-1 MS	100% Methyl Polysiloxane (100% Dimethylpolysiloxane) - low bleeding	350 °C	Yes	Low Bleed general purpose column for GC-MS. Solvent impurities, PCBs, Simulated Distillation, drugs, natural gases, hydrocarbons, essential oils, semivolatiles, pesticides, phenols	EPA: 504.1, 505, 606 USP: G1, G2, G9, G38
HI-1 PONA	100% Methyl Polysiloxane (100% Dimethylpolysiloxane) - optimized for hydrocarbon analysis	350 °C	Yes	Optimized for DHA (Detailed Hydrocarbons Analysis), PONA, PIANO and PNA analysis	ASTM D6730-01
HI-JXR	100% Methyl Polysiloxane	350 °C	Yes	General Purpose Apolar Column	USP: G1, G2, G9, G38
HI-SE30	100% Methyl Polysiloxane	350 °C	Yes	General Purpose Apolar Column	EPA: 504.1, 505, 606, 8141A USP: G1, G2, G9, G38
HI-PS255	1% Vinyl, 99% Methyl Polysiloxane	350 °C	Yes	Apolar phase to analyze solvents, alcohols, volatiles, suited to high film thicknesses	-

2. Aim of analysis/Application – App. Notes

Check available resources -

1. Avantor GC phase document and cross reference document.

2. Check application notes, check what are the trends of columns used - https://uk.vwr.com/cms/chromatography_chrom_library

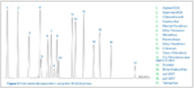
Active Filter(s)
- x Separation Mode: Gas Chromatography
- x Column Manufacturer: Hichrom
- x Remove All Filters

SEARCH APPLICATIONS

Free text: Search for... Go

Filter Options: Separation Mode: | Column Manufacturer: | Method: | Application Area: | Technique: | Substance...

Fast Separation of Pesticides using the Avantor® Hichrom HI-SE54 Phase



APPLICATION DETAILS

REQUEST MORE INFO

Separation Mode: Gas Chromatography
Method: -
Substance: Alpha-HCH; Gamma-HCH; Chlorothalonil; Heptachlor; Methyl Parathion; Ethyl Paraoxon; Malathion; Fenitrothion; Ethyl Parathion; Trans-Chlordane; Cis-Chlordane and Alpha-Endrin; Dieldrin; Beta-Endosulfan; o,p'-DDT; p,p'-DDT; Tetradifon
Matrix: -
Technique: GC
Column Manufacturer: Hichrom
Column Description: Avantor® Hichrom HI-SE54 GC Column, 0.10... (more)
Article No: HI05-10-010-5
System Used: -
Application Area: Pesticide / Herbicide

Abstract: A GC method for the fast separation of pesticides using the HI-SE54 phase.

44 Applications

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HI-PS255	1% Vinyl, 99% Methyl Polysiloxane	350 °C	Yes	Apolar phase to analyze solvents, alcohols, volatiles, suited to high film thicknesses	-

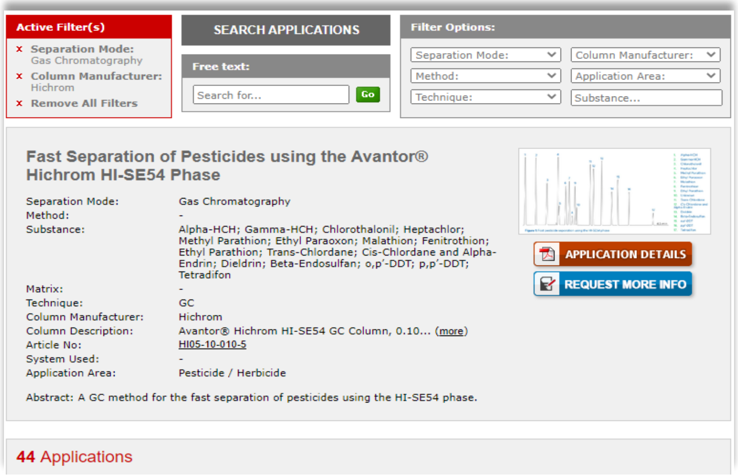
2. Aim of analysis/Application - Monographs

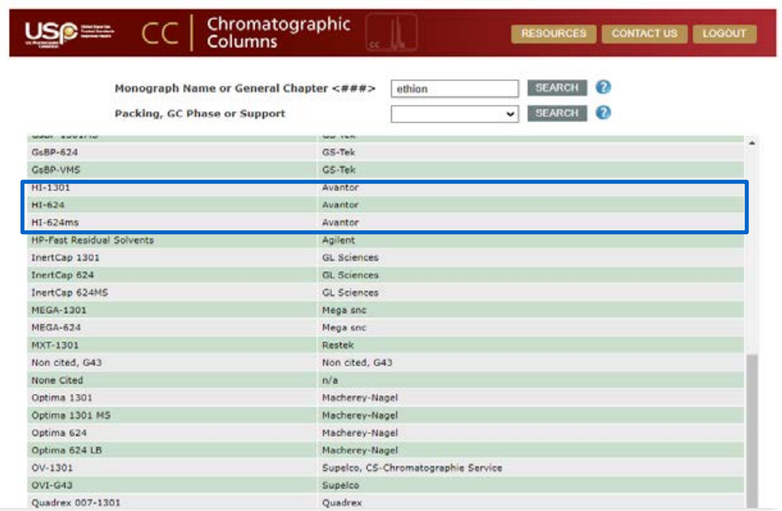
Check available resources -

1. Avantor GC phase document and cross reference document.

2. Check application notes, check what are the trends of columns used - https://uk.vwr.com/cms/chromatography_chrom_library

3. Check similar monograph/analyte or similar monograph of interest in USP databases - https://www.uspchromcolumns.com/





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3. GC configuration

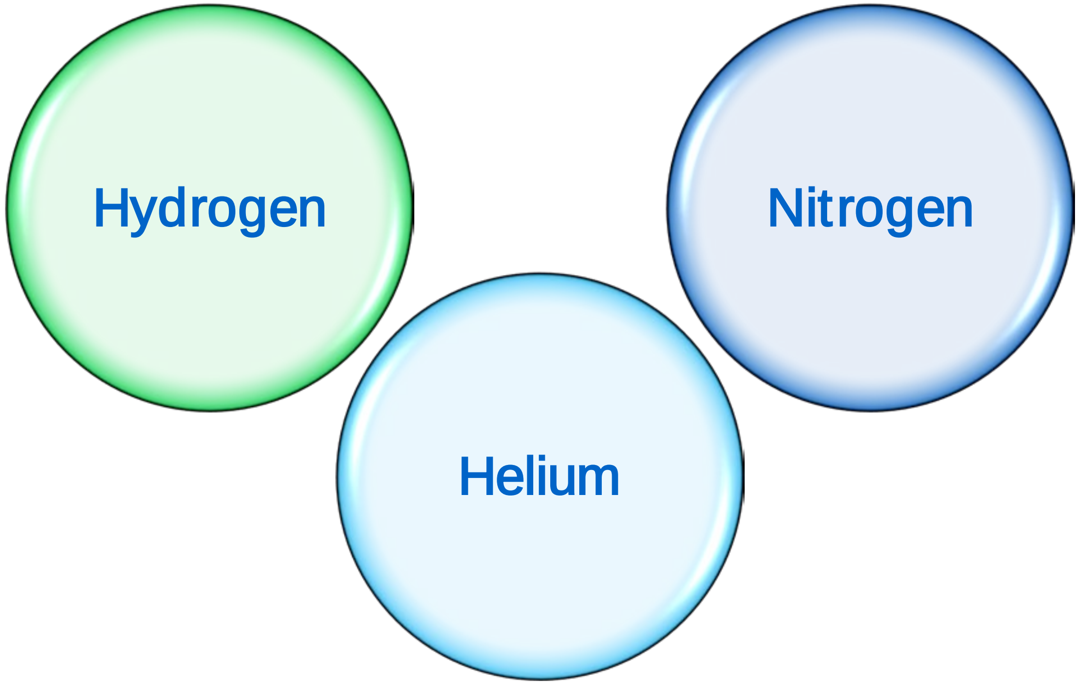
- Analytes of interest
- Aim of analysis/Application
- GC configuration

What is the GC configuration, gases, inlet, detector, samplers, GC type?

Is the GC configuration suitable for aim of analysis?

3. GC configuration – choice of carrier gas

Air is not suitable as a carrier gas!



Hydrogen
- Most efficient carrier gas
- Least viscous
- Highest diffusivity of the 3 gases

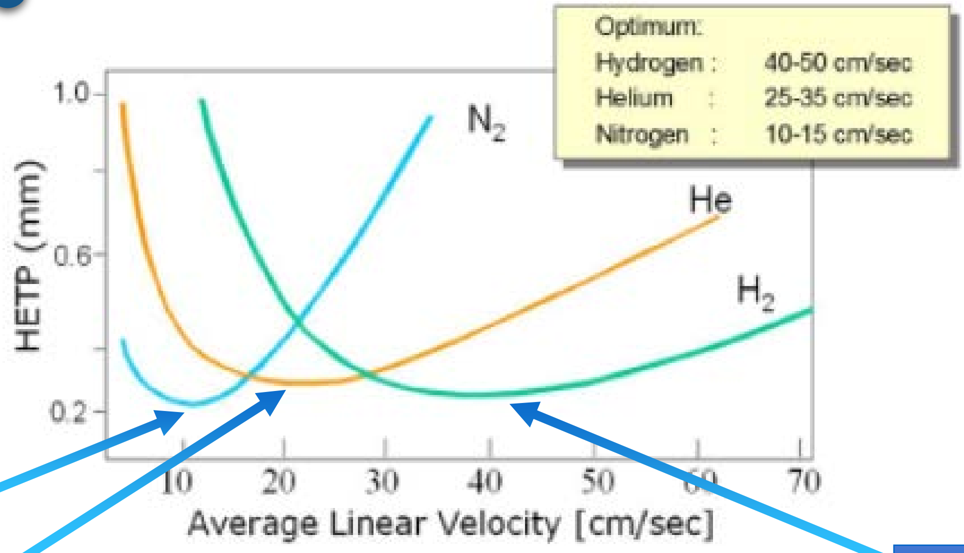
Nitrogen
- Originally used with packed GC columns
- 2nd most viscous
- Low diffusivity

Helium
- Most popular carrier gas
- Most viscous of the 3 gases
- Higher diffusivity than nitrogen

Gas qualities such as viscosity and diffusivity affect gas speed and pressure.

3. GC configuration - Carrier gas linear velocity

Q: Why does carrier gas linear velocity (speed cm/sec) matter?



Van Deemter equation*

*https://www.restek.com/globalassets/pdfs/literature/Impact-of-GC-Parameters_Part6.pdf

HETP (height equivalent to a theoretical plate)
HETP is a theoretical way to measure column efficiency, lower the better.

The faster the gas travels, gas linear velocity (speed cm/sec), the shorter the RT i.e. shorter analysis time.

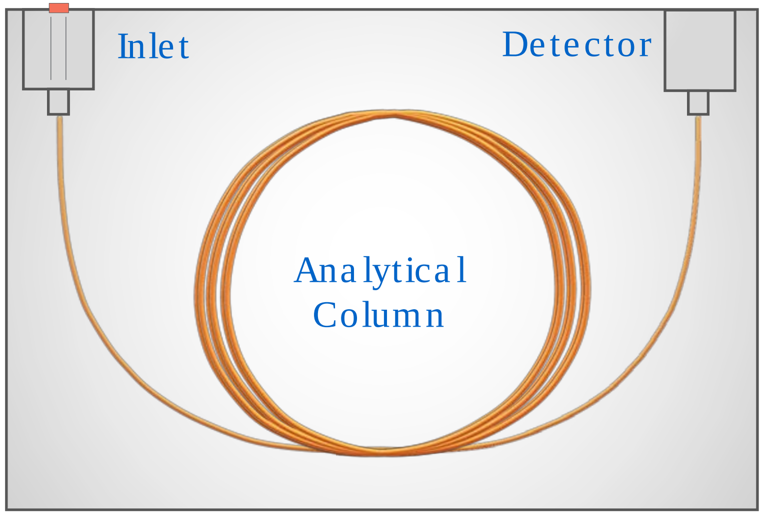
Carrier gas linear velocity
Too high = rapid RT but less resolution
Too low = long RT but more resolution

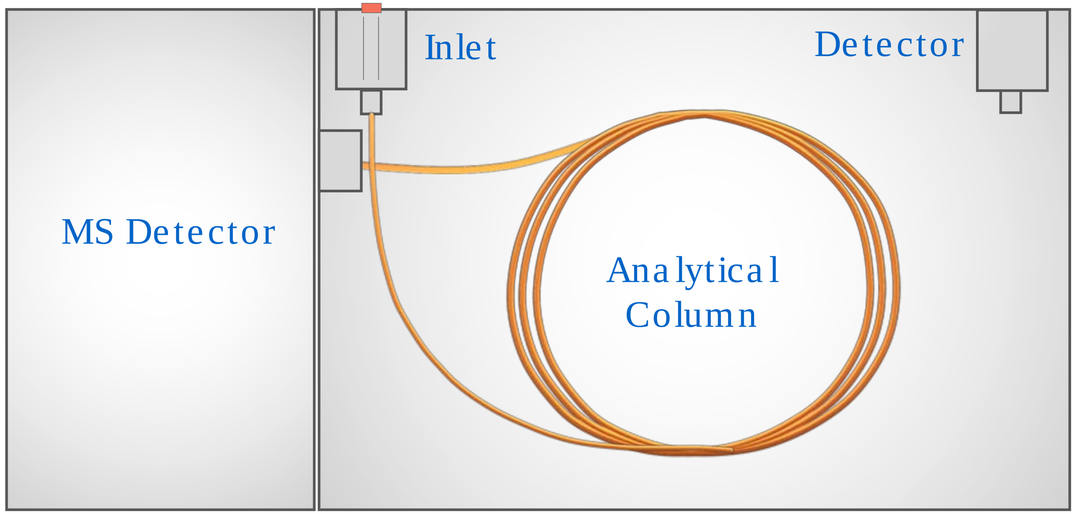
The optimum gas velocity is the balance between fast RT and good resolution.

3. GC configuration – Inlet and detector limits

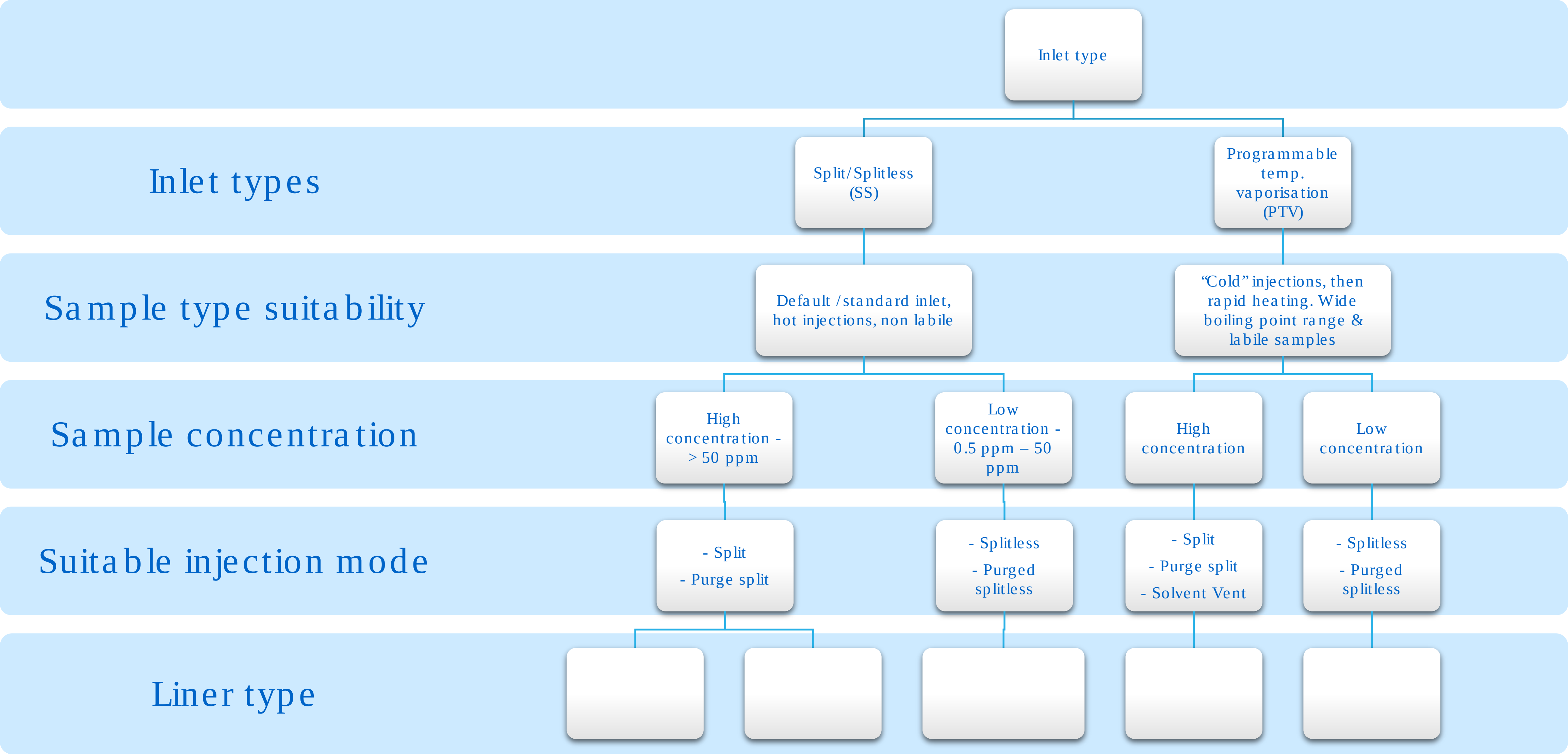
- Split/Splitless (SS)
- Cool-on-column (COC)
- Programmable Temperature Vaporization (PTV)
- Multimode inlet (MMI)
- Volatiles Interface (VI)

- Flame ionization detector (FID)
- Thermal conductivity detector (TCD)
- Flame photometric detector (FPD)
- Electron capture detector (ECD)
- Sulfur chemiluminescence detector (SCD)
- Mass Spectrometry (MS)

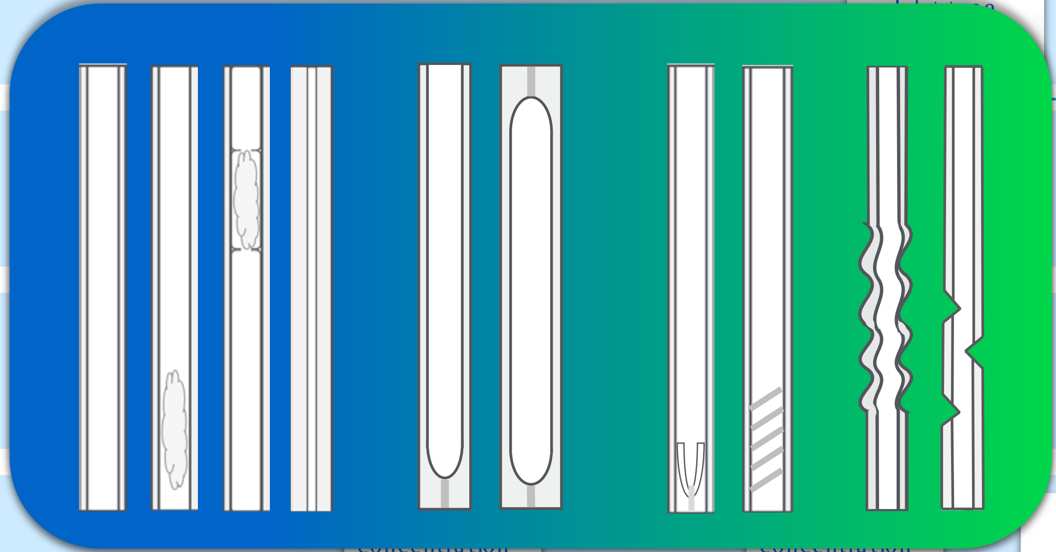




3. GC configuration – Inlet type



3. GC configuration – Inlet type and liners



Inlet types				Programmable temp. vaporisation (PTV)	
Sample type suitability				"Cold" injections, then rapid heating. Wide boiling point range & labile samples	
Sample concentration	50 ppm - % -		0.5 ppm – 50	High concentration	Low concentration
Suitable inject…	- Purge split		splitless	- Solvent Vent	- Splitless - Purged splitless
Liner type	Straight – With or without quartz wool	Straight Cyclo	Gooseneck or double gooseneck - With or without quartz wool	PTV or MMI – Baffled or dimpled	PTV or MMI – Baffled or dimpled

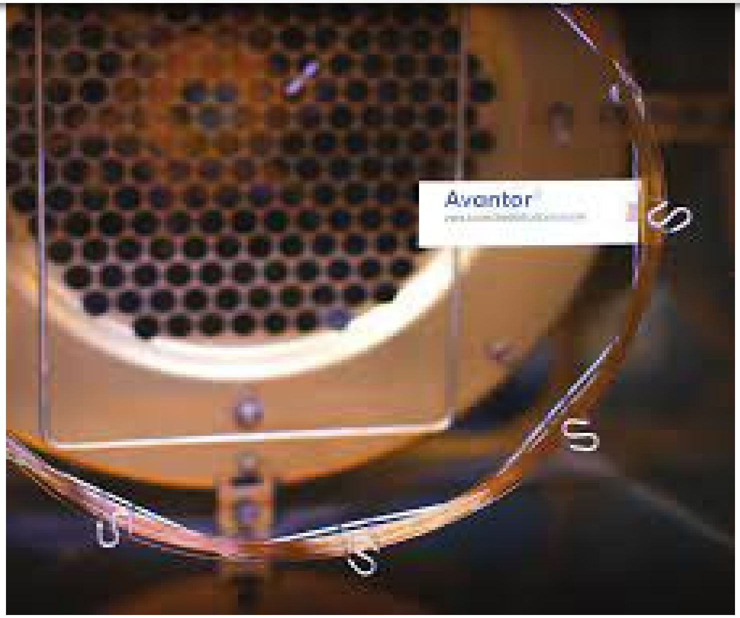
Ensure the liner is suitable for sample, inlet and sampler.
Consider vapour volume – Calculate vapour volume of solvent.

4. Column Phase

What column phase will retain and separate the analytes to fit the desired results?

What dimensions will be most suitable for my sample?

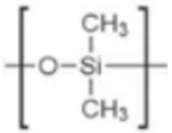
- Analytes of interest
- Aim of analysis/Application
- GC configuration
- GC column phase



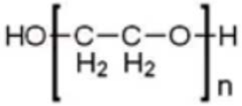
4. Column Phase - Most common column phase chemistries

Stationary phase is a liquid coating of Polysiloxanes or Polyethylene glycol with various substituent groups.

100% Dimethylpolysiloxane



Polyethylene glycol (PEG) or wax



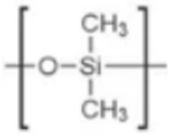
Stationary phase coating

Nonpolar — Polar/high polarity

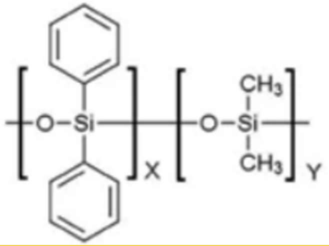
4. Column Phase - Most common column phase chemistries

To increase the polarity, the polysiloxane phase methyl groups are replaced by phenyls, cyanopropyl or other groups.

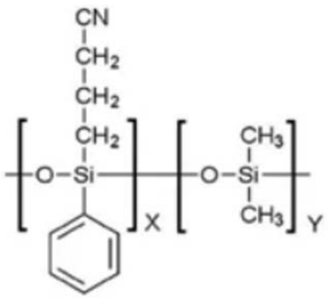
100% Dimethylpolysiloxane



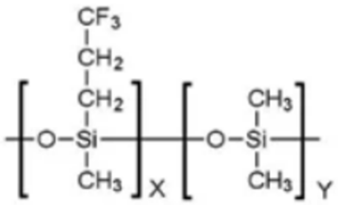
5% Phenyl, 95% Methyl Polysiloxane



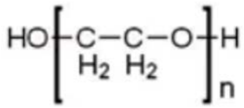
6% Cyanopropylphenyl, 94% Methyl Polysiloxane



Trifluoropropyl Methyl Polysiloxane



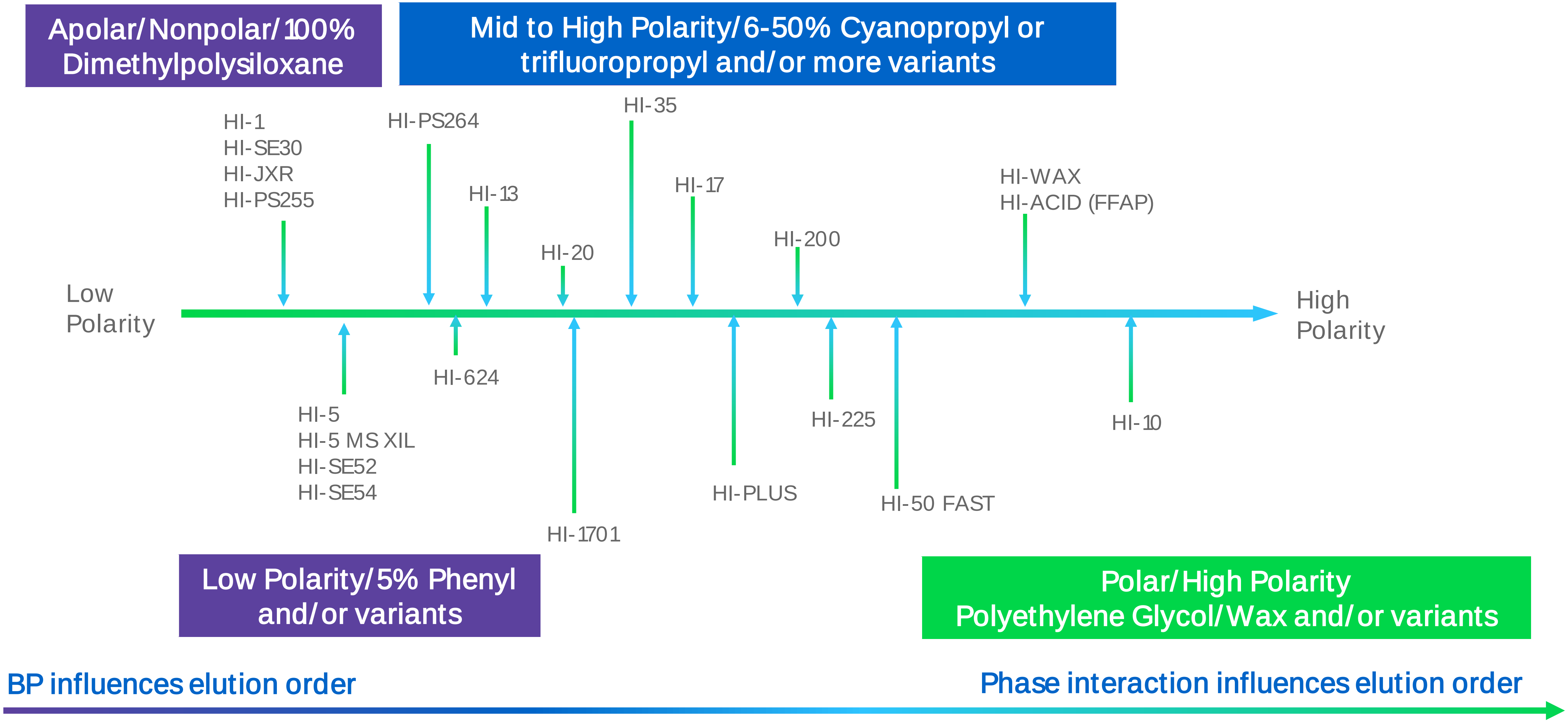
Polyethylene glycol (PEG) or wax



Stationary phase coating

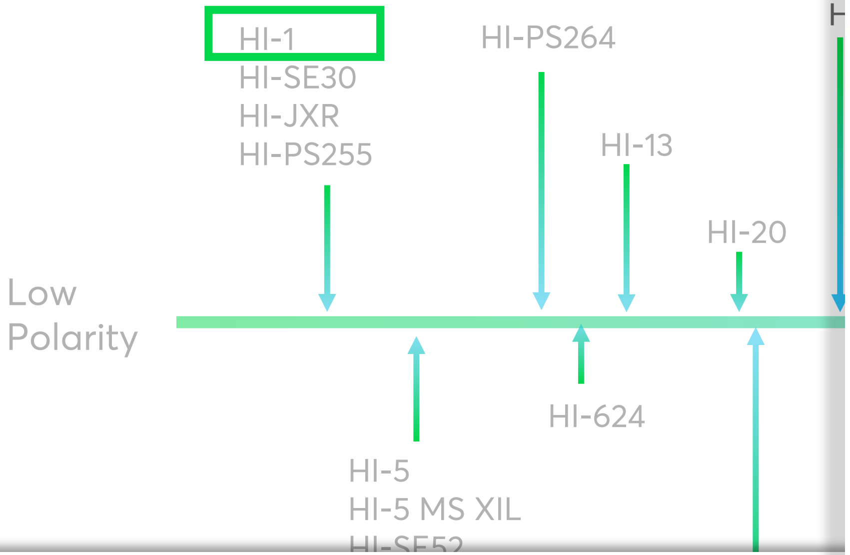
Nonpolar → Polar/high polarity

4. Column Phase - Polarity and separation mechanisms



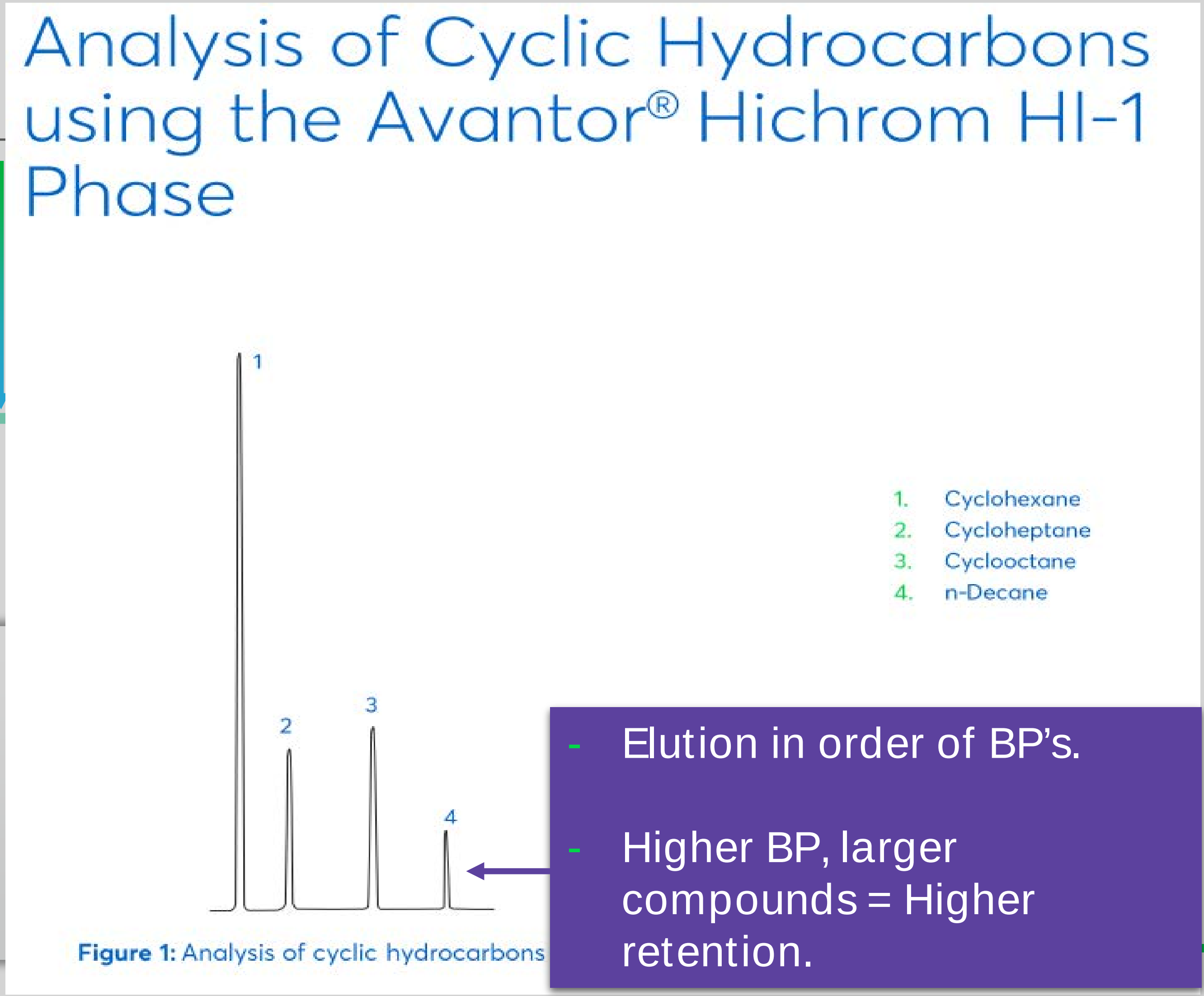
4. Column Phase - Polarity and separation mechanisms

Apolar/Nonpolar/100% Dimethylpolysiloxane



Example – Application note # C-13098

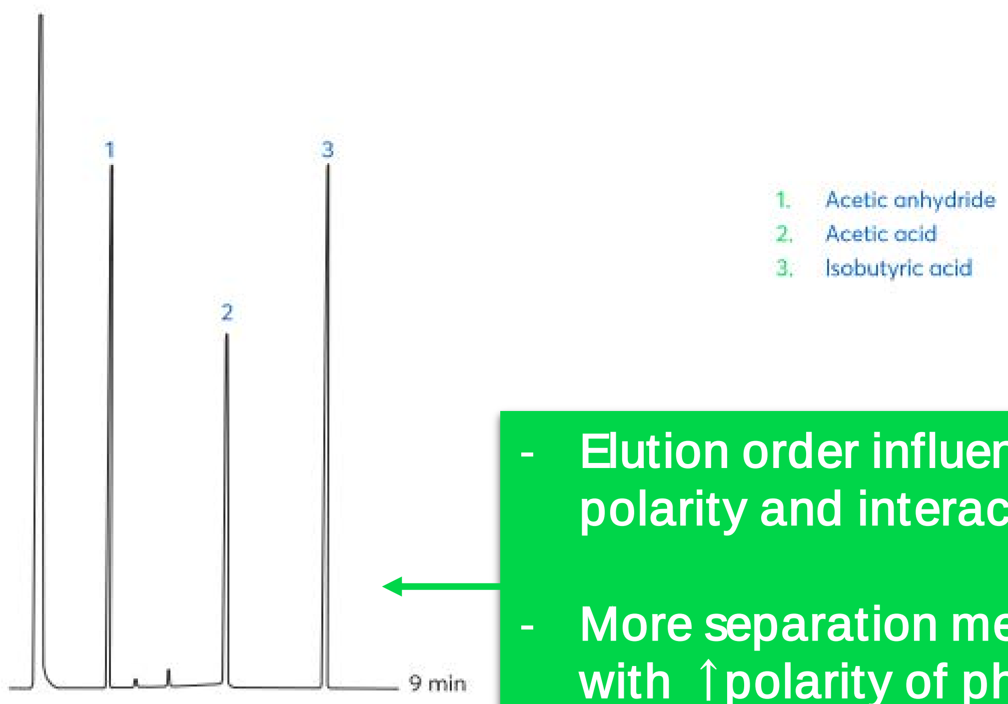
1. Cyclohexane – Non-polar, BP 80.75 °C
2. Cycloheptane – Non-polar, BP 118.4 °C
3. Cyclooctane - Non-polar, BP 149 °C
4. n-Decane - Non-polar, BP 174.1 °C

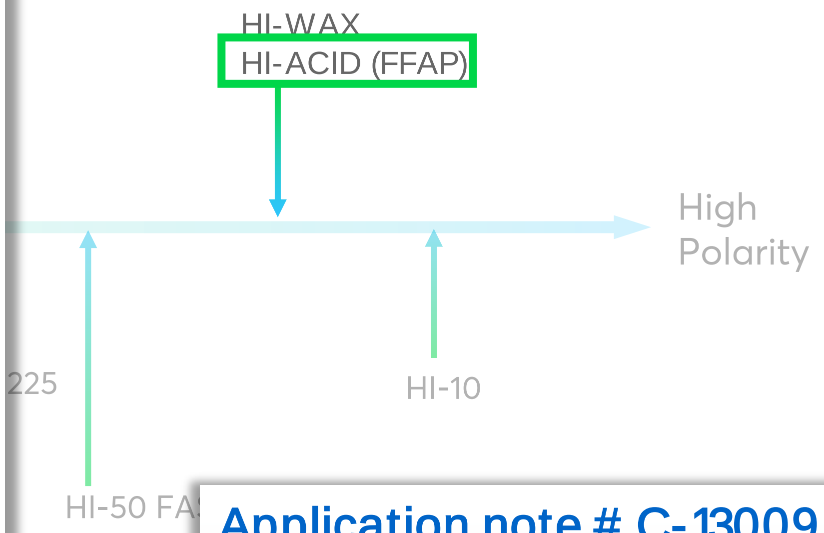


- Elution in order of BP's.
- Higher BP, larger compounds = Higher retention.

4. Column Phase - Polarity and separation mechanisms

Separation of Volatile Acidic Compounds using the Avantor® Hichrom HI-ACID (FFAP) Phase





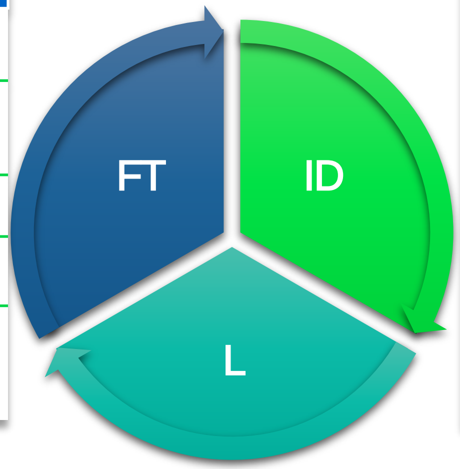
- Elution order influenced by polarity and interactions.
- More separation mechanisms with ↑polarity of phase.

Application note # C-13009

1. Acetic Anhydride – Polar, BP 139.5 °C
2. Acetic Acid – Polar, BP 118 °C
3. Isobutyric Acid – Polar, BP 155 °C

4. Column Phase – Column dimension considerations

Film Thickness	
Thin FT 0.10–0.50 µm	Thick FT 1–10 µm
Decreased retention and short RT	Increased Retention and longer RT
Lower sample capacity	Higher sample capacity
Higher temperatures	Lower Temperatures
Low column bleed	High column bleed
Medium to high molecular weight compounds	Volatiles and low molecular weight compounds



Column ID		
0.10-0.18 mm	0.25-0.32 mm	0.40-0.53 mm ID
Short RT	Moderate RT	Long RT
Low flow	Moderate flow	High flow
Lower sample capacity, <50 ng (based on 0.25 µm FT)	Medium sample capacity, <200 ng (based on 0.25 µm FT)	Higher sample capacity, < 2000 ng (based on 0.25 µm FT)
Split mode, Fast GC, GCMS, highly complex samples	Complex samples, split, splitless, DI, HS and on-column modes, broad conc. range.	Split, splitless, DI, HS and on-column modes.

Column Length		
Short <15 m	Medium 20-30 m	60-100 m
Lower resolution	Medium resolution, suits broad range	Increased Resolution
Short RT	Moderate RT	Long RT
Lower cost	Medium cost, more popular, general use length at 30 m	Higher cost, consider other options before increasing length
A few compounds in sample, high boilers, Fast GC, GCMS	Medium complexity of samples, GCMS	Very complex samples, low boilers

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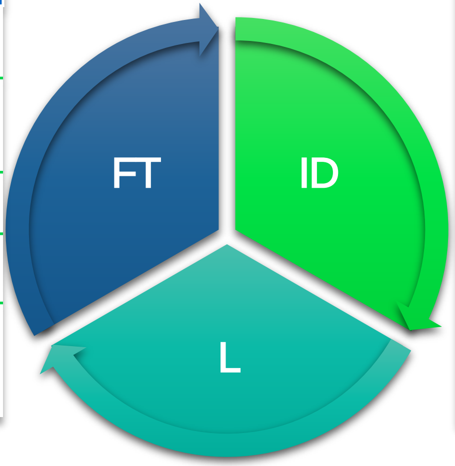

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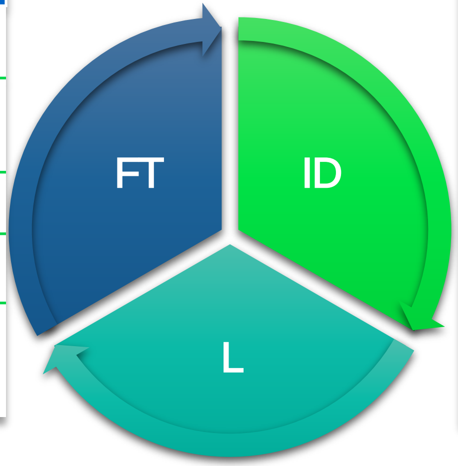
Low column bleed	High column bleed
Medium to high molecular weight compounds	Volatiles and low molecular weight compounds



Column ID		
0.10-0.18 mm	**0.25-0.32 mm**	**0.40-0.53 mm ID**
Short RT	Moderate RT	Long RT
Low flow	Moderate flow	High flow
Lower sample capacity, <50 ng (based on 0.25 µm FT)	Medium sample capacity, <200 ng (based on 0.25 µm FT)	Higher sample capacity, < 2000 ng (based on 0.25 µm FT)
Split mode, Fast GC, GCMS, highly complex samples	Complex samples, split, splitless, DI, HS and on-column modes, broad conc. range.	Split, splitless, DI, HS and on-column modes.

Column Length		
Short <15 m	**Medium 20-30 m**	**60-100 m**
Lower resolution	Medium resolution, suits broad range	Increased Resolution
Short RT	Moderate RT	Long RT
Lower cost	Medium cost, more popular, general use length at 30 m	Higher cost, consider other options before increasing length
A few compounds in sample, high boilers, Fast GC, GCMS	Medium complexity of samples, GCMS	Very complex samples, low boilers

5. Sample mixture

What is in the sample mixture?

Is there a sample preparation protocol ready?

Is the sample solvent/diluent compatible with the column?

- Analytes of interest
- Aim of analysis/Application
- GC configuration
- GC column phase
- Sample mixture

5. Sample mixture - GC sample solvent/diluent selection

Lower polarity sample analytes and columns

- Low polarity solvent, e.g. n-hexane.

Mixture of polarities and/or mid polarity columns

- An intermediate polarity solvent may be used to compromise, e.g. ethyl acetate.

Polar sample analytes and more polar columns

- Higher polar solvent, e.g. Methanol.

Other considerations

- Sampler type and detectors need to be considered.
- Solvent needs a lower boiling point than compounds in sample mixture.

Objectives, GC setup and aims established

- Analytes of interest
- Aim of analysis/Application
- GC configuration
- GC column phase
- Sample mixture

Goals of optimizing parameters

01

– Transfer enough sample onto column for detection

02

– See all peaks elute

03

– Reduce retention time

04

– See good peak shape and resolution of peaks

05

– See a good response to enable concentration calibration

06

– Achieve reproducible results

Goals of optimizing parameters

01

– Transfer enough sample onto column for detection

02

– See all peaks elute

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– Reduce retention time

04

– See good peak shape and resolution of peaks

05

– See a good response to enable concentration calibration

06

– Achieve reproducible results – **Once RT reduced, resolution is good, all peaks elute and decent responses, then run further injections to test reproducibility.**

Scout run

1st Approach – Application note located

2nd Approach - Set up method (default method or manually)

Scout run

1st Approach – Application note located

- Acquire suitable test mix/external standard.
- Application note available – Use method parameters if applicable.
- Adjust parameters to suit GC config.
- Run injection, assess results.

2nd Approach - Set up method (default method or manually)

Scout run

1st Approach – Application note located

- Acquire suitable test mix/external standard.
- Application note available – Use method parameters if applicable.
- Adjust parameters to suit GC config.
- Run injection, assess results.
- Set up more runs with adjusted parameters - E.g. Oven ramp 20°C, 30°C, 40 °C and 50 °C/min. Select best oven ramp, then adjust another parameter and run more injections.
- Parameter by parameter if possible.

2nd Approach - Set up method (default method or manually)

Scout run

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- Optimize parameters and set up more runs, adjust one by one.
- Parameter by parameter if possible.

Scout run

1st Approach – Application note located

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- Application note available – Use method parameters if applicable.
- Adjust parameters to suit GC config.
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- Parameter by parameter if possible.

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- Run injection, assess results.
- Set up more runs with adjusted parameters - E.g. Oven ramp 20 °C, 30 °C, 40 °C and 50 °C/min. Select best oven ramp, then adjust another parameter and run more injections.
- Optimize parameters and set up more runs, adjust one by one.
- Parameter by parameter if possible.

Avoid changing all parameters at once!

First run, split injection

Method starting point with 0.32 mm X 25 m column using SS inlet

Split injection starting parameters	
Column capacity	50 – 150 ng per analyte, use higher end of capacity, 0.32 mm X 25 m
Injection volume	1 µL, e.g. 150 ng/µL
Inlet temp	250 °C
Column flow	1 ml/min (0.9 - 1.8 mL/min)
Column flow mode	Constant flow
Split ratio	50:1
Initial temp	40 °C
Initial hold time	NA
Oven ramp rate	10 °C/min
Final temp	Max operating temp of column if needed e.g. 360 °C, - 10 - 20 °C
Final hold time	10 min

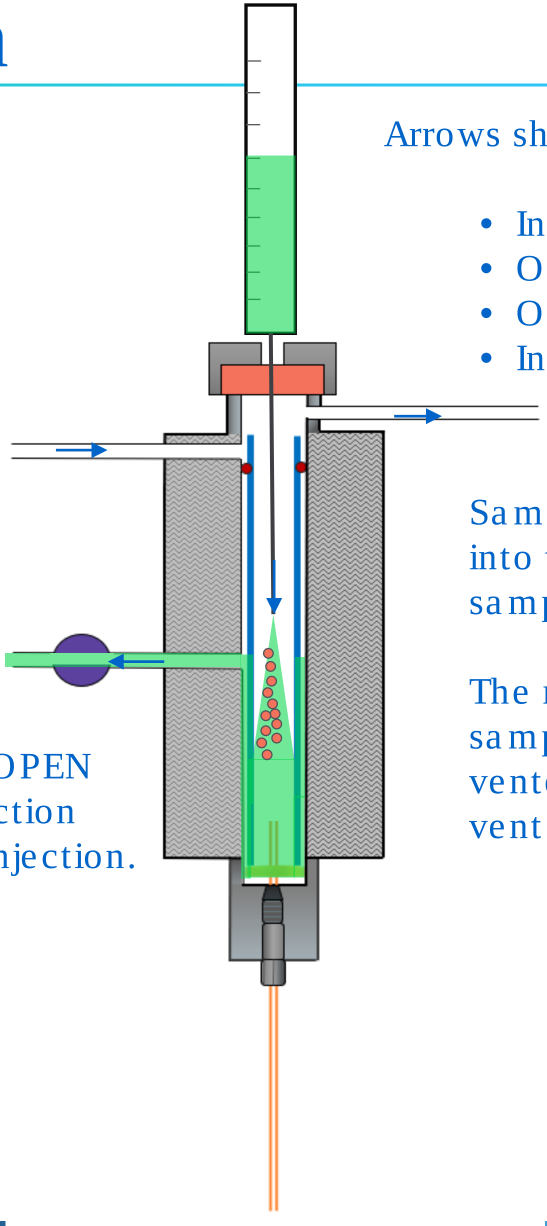
GC inlet – Split injection

Used when the sample **concentration is too high**.

Splits off the majority of the volatilized sample and adjusts the amount of sample transferred to the column.

Arrows show carrier gas flow –

- Into inlet
- Out of septum purge
- Out the split vent
- Into column



Split vent line/valve OPEN during injection and after injection.

Sample is introduced into the inlet and liner, sample is vaporised.

The majority of the sample and vapour is vented out the split vent line.

Optimized split injection – Adjust flows and inlet 1st

Split injection starting parameters		Optimize - run more injections with a number of adjusted parameters
Column capacity	50 – 150 ng per analyte, use higher end of capacity, 0.32 mm X 25 m	+/- 50 ng, as needed, see below first before adjusting sample concentration.
Injection volume	1µL, e.g. 150 ng/µL	Overload = Dilute sample if increasing split flow does not help. Low response = + 0.5 µL steps if decreasing split flow does not help.
Inlet temp	250 °C	+25°C steps up to 300°C, **if needed**, choose best temp (Too high = degradation).
Column flow	1 ml/min (0.9 - 1.8 mL/min)	+ 0.2 m L/min (0.9 - 1.8 mL/min) or increase linear velocity to by + 5 cm/sec steps.
Column flow mode	Constant flow	
Split ratio	1:50	Split of 1:75, 1:100, 1:150, 1:200 (can go higher if needed). Ensure enough sample is transferred to the column.
Initial oven temp	40 °C	
Initial oven hold time	NA in split	
Oven ramp rate	10 °C/min	
Final temp	Max operating temp of column if needed e.g. 360 °C, - 10 - 20 °C	
Final hold time	10 min	

Optimized split injection – Adjust oven ramp/temps. 2nd

Split injection starting parameters		Optimize - run more injections with a number of adjusted parameters
Column capacity	50 – 150 ng per analyte, use higher end of capacity, 0.32 mm X 25 m	+/- 50 ng, as needed, see below first before adjusting sample concentration.
Injection volume	1µL, e.g. 150 ng/µL	Overload = Dilute sample if increasing split flow does not help. Low response = + 0.5 µL steps if decreasing split flow does not help.
Inlet temp	250 °C	+25°C steps up to 300°C, **if needed**, choose best temp (Too high = degradation).
Column flow	1 ml/min (0.9 - 1.8 mL/min)	+ 0.2 m L/min (0.9 - 1.8 mL/min) or increase linear velocity to by + 5 cm/sec steps.
Column flow mode	Constant flow	
Split ratio	1:50	Split of 1:75, 1:100, 1:150, 1:200 (can go higher if needed). Ensure enough sample is transferred to the column.
Initial oven temp	40 °C	Calculate T(i) (oven temperature of 1st eluting peak) T initial = T(i) – 45 °C.
Initial oven hold time	NA in split	Add hold for mid eluters, If needed, hold temperature over coeluting analytes.
Oven ramp rate	10 °C/min	Optimum Ramp Rate = 10 °C per t_0. Steps of + 20 °C, 30 °C, 40 °C and 50 °C/min.
Final temp	Max operating temp of column if needed e.g. 360 °C, - 10 - 20 °C	Check T(f) (final analyte elution temp), then calculate final temp(T (f) = T(f) + 20 °C, as long as it does not exceed max temperature of column.
Final hold time	10 min	Reduce or remove (only needed if all analytes not eluted and max temp reached).

First run, splitless injection

Method starting point with 0.32 mm X 25 m column using SS inlet

Splitless injection starting parameters	
Column capacity	50 – 150 ng per analyte, use higher end of capacity, 0.32 mm X 25 m
Injection volume	1 µL, e.g. 150 ng/µL
Inlet temp	250 °C
Column flow	1 mL/min (0.9 - 1.8 mL/min)
Column flow mode	Constant flow
Splitless hold time	1 min (2 min is usually the maximum time), or time for the above.
Splitless purge flow	Common default is 50 mL/min
Initial temp	20 °C below BP of the solvent or as low as possible.
Initial hold time	Match to splitless hold time (up to 2 minutes)
Oven ramp rate	10 °C/min
Final temp	Max operating temp of column if needed e.g. 360 °C, -10-20 °C
Final hold time	10 min

Splitless is different to split in oven parameters

Initial temp is 20 °C below BP of the solvent or as low as possible to allow -

1. Solvent focusing

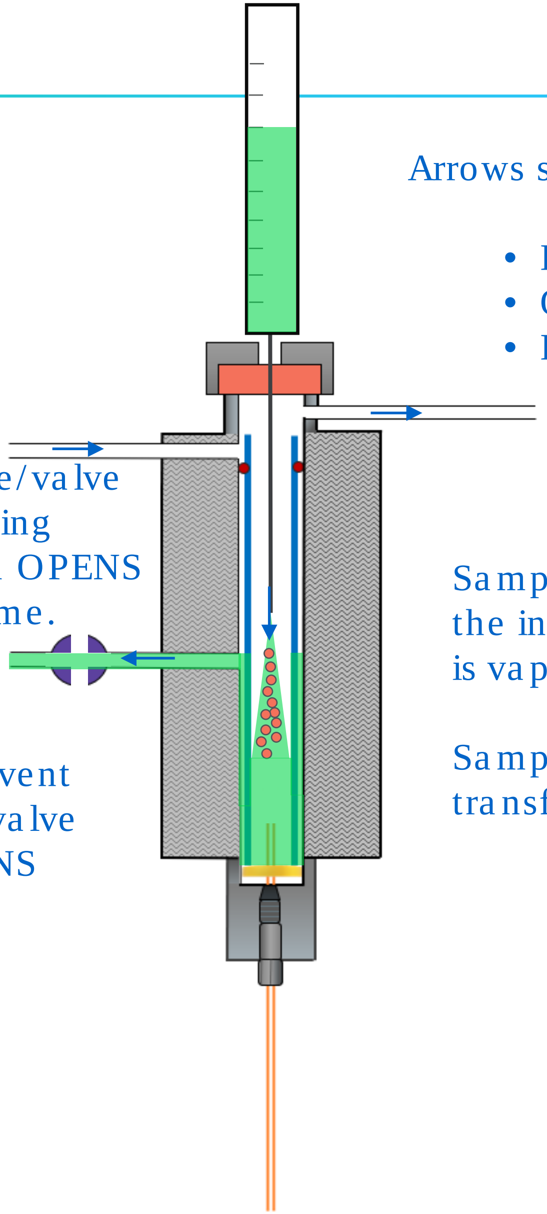
2. Cold trapping

GC inlet – Splitless injection

All of the sample is transferred to the column and suitable for low concentration samples.

Transfer of sample vapour is much slower, up to 2 minutes.

The split line opens at an optimized purge time to clear the inlet of any residual vapours.



Optimize splitless injection - Adjust flows and inlet 1st

Splitless injection starting parameters		Optimize parameters for more runs based on results of scout run
Column capacity	50 – 150 ng per analyte, use higher end of capacity, 0.32 mm X 25 m	+/- 50 ng, as needed, see below first before adjusting sample concentration.
Injection volume	1 µL, e.g. 150 ng/µL	Overload = Dilute sample if increasing split flow does not help. Low response = + 0.5 µL steps if decreasing split flow does not help.
Inlet temp	250 °C	+25°C steps up to 300°C, **if needed**, choose best temp (Too high = degradation).
Column flow	1 ml/min (0.9 - 1.8 mL/min)	+ 0.2 m L/min (0.9 - 1.8 mL/min) or increase linear velocity to by + 5 cm/sec steps.
Column flow mode	Constant flow	
Splitless hold time	1 min (2 min is usually the maximum time), or time for the above.	Adjust 1.5 to 2 times carrier gas sweep of the total inlet, up to 2 min.
Splitless purge flow	Common default is 50 mL/min	+ steps 10 mL/min, but only adjust as a last resort if there is issues with carryover.
Initial temp	20 °C below BP of the solvent or as low as possible.	
Initial hold time	Match to splitless hold time (up to 2 minutes)	
Oven ramp rate	10 °C/min	
Final temp	Max operating temp of column if needed e.g. 360 °C, -10-20 °C	
Final hold time	10 min	

Optimize splitless injection - Adjust oven ramp/temps. 2nd

Splitless injection starting parameters		Optimize parameters for more runs based on results of scout run
Column capacity	50 – 150 ng per analyte, use higher end of capacity, 0.32 mm X 25 m	+/- 50 ng, as needed, see below first before adjusting sample concentration.
Injection volume	1µL, e.g. 150 ng/µL	Overload = Dilute sample if increasing split flow does not help. Low response = + 0.5 µL steps if decreasing split flow does not help.
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Column flow mode	Constant flow	
Splitless hold time	1 min (2 min is usually the maximum time), or time for the above.	Adjust 1.5 to 2 times carrier gas sweep of the total inlet, up to 2 min.
Splitless purge flow	Common default is 50 mL/min	+ steps 10 mL/min, but only adjust as a last resort if there is issues with carryover.
Initial temp	20 °C below BP of the solvent or as low as possible.	Reduce if peaks widening. Reassess after another run. Check bp of solvent is suitable for splitless injection.
Initial hold time	Match to splitless hold time (up to 2 minutes)	Match to splitless hold time (up to 2 minutes), add hold for mid eluters, If needed, hold temperature over coeluting analytes.
Oven ramp rate	10 °C/min	Optimum Ramp Rate = 10 °C per t_0. Steps of + 20 °C, 30 °C, 40 °C and 50 °C/min.
Final temp	Max operating temp of column if needed e.g. 360 °C, - 10 - 20 °C	Check T(f) (final analyte elution temp), then calculate final temp(T (f) = T(f) + 20 °C, as long as it does not exceed max temperature of column.
Final hold time	10 min	Reduce or remove (only needed if all analytes not eluted and max temp reached).

Fast GC

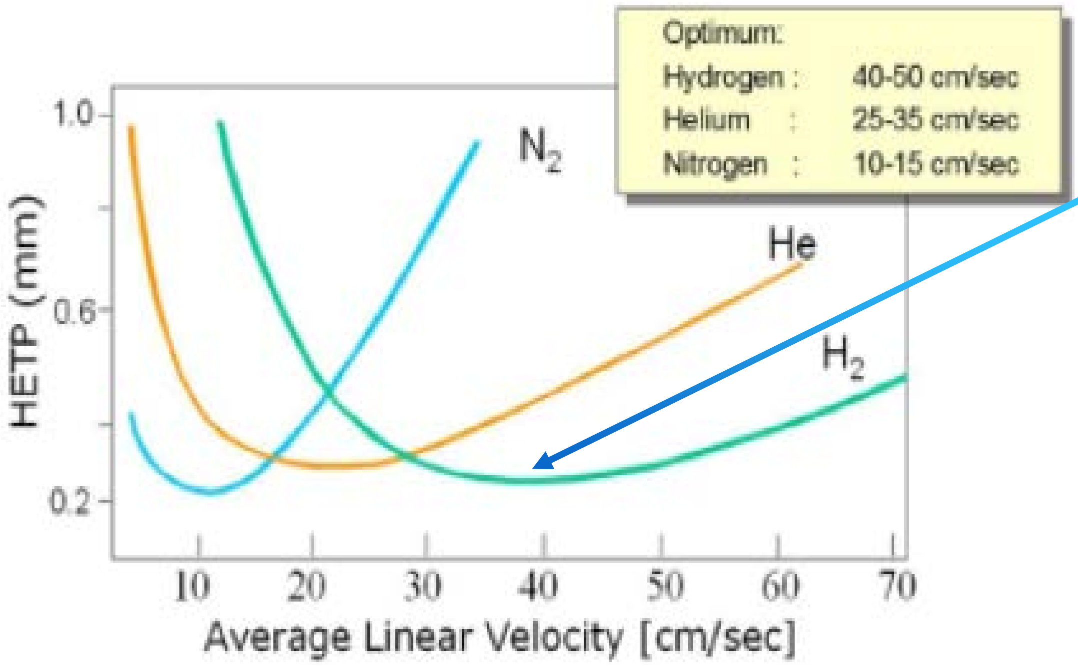
What is Fast GC?

It is a technique that allows you to reduce the analysis time while keeping an adequate resolution power, thus increasing your throughput.

Can be applied to medium-to-high complexity mixtures analysis.

Provides 3–10 times faster analysis compared to conventional GC. Great for screening analysis.

Fast GC – What is required?



*https://www.restek.com/globalassets/pdfs/literature/Impact-of-GC-Parameters_Part6.pdf

What you need to make FAST-GC

To reduce retention time -

- Length - Shorter column 5 - 10 m.
- High temperature ramp (usually more than 15°C/min).
- Higher gas linear velocity.

To accommodate for decrease in resolution -

- Use H_2 carrier gas – optimum gas velocity (fastest).
- ID - Smaller ID, usually 0.10 mm.
- FT - 0.05 - 0.20 µm.

Also requires -

- Fast acquisition rates - frequency of at least 50Hz.

Additional information -

- Old MS systems may not be able to handle Hydrogen as a carrier gas.

Fast GC – GC Considerations

https://www.agilent.com/cs/library/slidepresentation/public/Fast_GC_Methods_ISCC_Agilent_2017.pdf

Agilent Intuvo 9000 GC instrument

The Intuvo GC oven has a smaller, compact design and special, unique GC columns have to be installed.

Avantor do not supply these GC capillary column formats. **Standard GC or FAST GC capillary columns are NOT compatible with the Agilent Intuvo 9000 GC.**

Standard GC and Fast GC capillary columns, example shown in the image on the bottom left, can be used on other standard GC instruments.

Transfer from standard to Fast GC

A shorter column will show a faster RT without changing any parameters. But the method needs to be optimized.

Transfer from standard to Fast GC – Standard method

Main adjustments to method

1 Increase carrier gas velocity

2. Adjust inlet flows - is enough sample transferring to column?

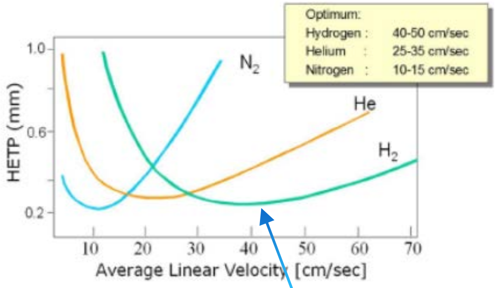
3. Adapt oven ramp rate

Method parameters	Original method
Column Length	30 m
Column ID	0.25 mm
Column FT	0.50 µm
Carrier gas	Helium
Linear velocity	42 cm/sec
Column flow	2 mL/min
Flow mode	Constant flow
Split ratio	1:10
Initial temp	40 °C
Initial hold time	0 min
Oven ramp rate	10 °C/min
Final temp	270 °C
Final hold time	2 min
Run time	25 min

Transfer from standard to Fast GC - Carrier gas velocity

1. Increase carrier gas velocity

- Faster carrier gas velocity decreases the RT.
- From 40 cm/sec, -/+ 10 cm/sec steps, 50, 60 cm/sec.
- Should see ↓ RT by a few min. to ½ original RT at least.



The optimum gas velocity is at the lowest point of the curve.

*https://www.restek.com/globalassets/pdfs/literature/Impact-of-GC-Parameters_Part6.pdf

2. Adjust inlet flows - is enough sample transferring to column?

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2. Adjust inlet flows - is enough sample transferring to column?

3. Adapt oven ramp rate

Method parameters	Original method	Fast GC method
Column Length	30 m	10 m
Column ID	0.25 mm	0.18 mm
Column FT	0.50 µm	0.40 µm
Carrier gas	Helium	Hydrogen
Linear velocity	42 cm/sec	80 cm/sec
Column flow	2 mL/min	1.35 mL/min
Flow mode	Constant flow	Constant flow or velocity
Split ratio	1:10	
Initial temp	40 °C	
Initial hold time	0 min	
Oven ramp rate	10 °C/min	
Final temp	270 °C	
Final hold time	2 min	
Run time	25 min	

Transfer from standard to Fast GC - Inlet flows

1 Increase carrier gas velocity

- Faster carrier gas velocity ↓ the RT.
- From 40 cm/sec, -/+ 10 cm/sec steps, 50, 60 cm/sec.
- Should see ↓ RT by a few min to ½ original RT at least.

2. Adjust inlet flows - is enough sample transferring to column?

- ↑ velocity = ↑ column flow and split flow.
- Poor peak shape due to overload = Split ratio too low.
- Poor response and peak loss = Split ratio too high.
- -/+ 25 - 50 to adjust split ratio, ↓ dimensions = min.
  1:100 - 1:400 (10x less sample conc. than standard GC).

3. Adapt oven ramp rate

-
-
-

Transfer from standard to Fast GC - Inlet flows

1. Increase carrier gas velocity

- Faster carrier gas velocity ↓ the RT.
- From 40 cm/sec, -/+ 10 cm/sec steps, 50, 60 cm/sec.
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- Poor peak shape due to overload = Split ratio too low.
- Poor response and peak loss = Split ratio too high.
- -/+ 25 - 50 to adjust split ratio, ↓ dimensions = min.
  1:100 - 1:400 (10x less sample conc. than standard GC).

3. Adapt oven ramp rate

-
-
-

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Carrier gas	Helium	Hydrogen
Linear velocity	42 cm/s	80 cm/s
Column flow	2 mL/min	1.35 mL/min
Flow mode	Constant flow	Constant flow or velocity
Split ratio	1:10	1:100 - 400
Initial temp	40 °C	
Initial hold time	0 min	
Oven ramp rate	10 °C/min	
Final temp	270 °C	
Final hold time	2 min	
Run time	25 min	

Transfer from Standard to Fast GC – Oven ramp rate

1 Increase carrier gas velocity

- Faster carrier gas velocity ↓ the RT.
- Start with 40 cm/sec, -/+ 10 cm/sec steps, 50, 60 cm/sec.
- Should see ↓ RT by a few min. to ½ original RT at least.

2. Adjust inlet flows - is enough sample transferring to column?

- ↑ velocity = ↑ column flow and split flow.
- Poor peak shape due to overload = Split ratio too low.
- Poor response and peak loss = Split ratio too high.
- -/+ 25 - 50 to adjust split ratio, ↓ dimensions = min. 1:100- 1:400 (10x less sample conc. than standard GC).

3. Adapt oven ramp rate

- + 10 °C/min, 20 °C, 30 °C, 40 °C and 50 °C/min (or ↑).
- Calculate optimum ramp rate = 10 °C per t_0.
- Further calculations for suitable oven ramp rate and isothermal temps. Can be used.

Transfer from Standard to Fast GC – Oven ramp rate

1 Increase carrier gas velocity

- Faster carrier gas velocity ↓ the RT.
- Start with 40 cm/sec, -/+ 10 cm/sec steps, 50, 60 cm/sec
- Should see ↓ RT by a few min. to ½ original RT at least.

2. Adjust inlet flows - is enough sample transferring to column?

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- Poor peak shape due to overload = Split ratio too low.
- Poor response and peak loss = Split ratio too high.
- -/+ 25 - 50 to adjust split ratio, ↓ dimensions = min. 1:100- 1:400. (10x less sample conc. than standard GC).

3. Adapt oven ramp rate

- + 10 °C/min, 20 °C, 30 °C, 40 °C and 50 °C/min (or ↑).
- Calculate optimum ramp rate = 10 °C per t_0.
- Further calculations for suitable oven ramp rate and isothermal temps. Can be used.

1. Oven ramp rate

$$t_{g2} = t_{g1} \frac{v_2}{v_1} \frac{\beta_2}{\beta_1} \frac{l_1}{l_2}$$

2. Isothermal hold time

$$T_2 = T_1 \frac{v_1}{v_2} \frac{\beta_1}{\beta_2} \frac{l_2}{l_1}$$

Where;
t_{g1}, t_{g2} – temp. gradient for orig. & new conditions
v_1, v_2 - linear velocity of gas for orig. & new conditions
T_1, T_2 - Isothermal hold time for orig. & new conditions
β_1, β_2 - Phase ratio for orig. & new conditions
l_1, l_2 - Length of column for orig. & new conditions

Optimisation of Column Parameters in GC- Peter Morgan, Anila Khan, Tony Edge – Thermo Scientific, Runcorn, UK

Transfer from Standard to Fast GC – Oven ramp rate

1 Increase carrier gas velocity

- Faster carrier gas velocity ↓ the RT.
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- 1:100- 1:400 (10x less sample conc. than standard GC).

3. Adapt oven ramp rate

- + 10 °C/min, 20 °C, 30 °C, 40 °C and 50 °C/min (or ↑).
- Calculate optimum ramp rate = 10 °C per t_0.
- Further calculations for suitable oven ramp rate and isothermal temps. Can be used.

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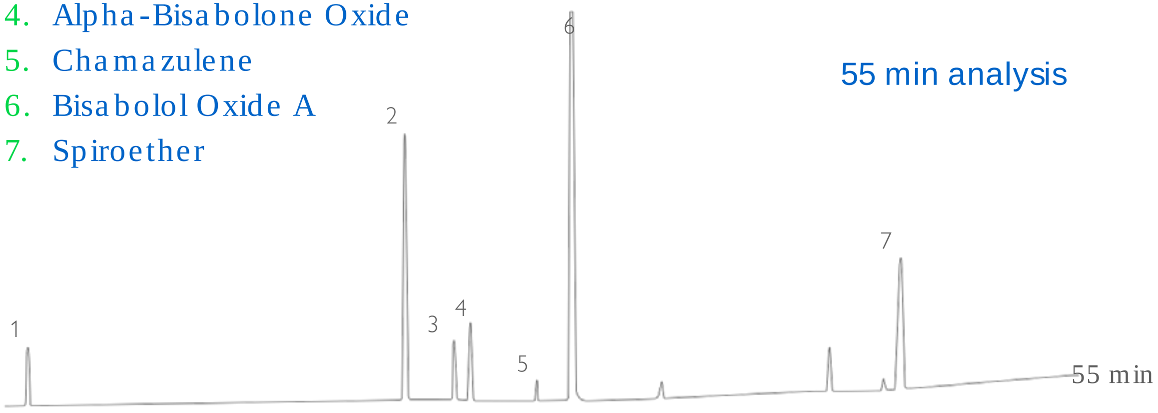
Column ID	0.25 mm	0.18 mm
Column FT	0.50 µm	0.40 µm
Carrier gas	Helium	Hydrogen
Linear velocity	42 cm/s	80 cm/s
Column flow	2 mL/min	1.35 mL/min
Flow mode	Constant flow	Constant flow or velocity
Split ratio	1:10	1:100 - 400
Initial temp	40 °C	120 °C
Initial hold time	0 min	0 min
Oven ramp rate	10 °C/min	30 °C/min
Final temp	270 °C	270 °C
Final hold time	2 min	1 min
Run time	25 min	

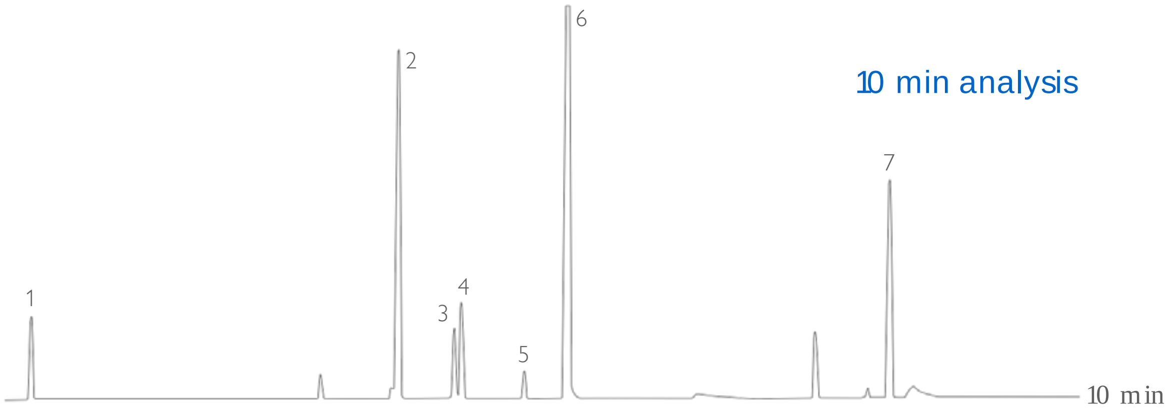
Transfer from Standard to Fast GC – New analysis time

Method parameters	Original method	Fast GC method
Column Length	30 m	10 m
Column ID	0.25 mm	0.18 mm
Column FT	0.50 µm	0.40 µm
Carrier gas	Helium	Hydrogen
Linear velocity	42 cm/s	80 cm/s
Column flow	2 mL/min	1.35 mL/min
Flow mode	Constant flow	Constant flow or velocity
Split ratio	1:10	1:100 - 400
Initial temp	40 °C	120 °C
Initial hold time	0 min	0 min
Oven ramp rate	10 °C/min	30 °C/min
Final temp	270 °C	270 °C
Final hold time	2 min	1 min
Run time	25 min	6 min

Comparison of Standard and FAST GC for Chamomile Analysis

1. Trans-Beta-Farnesene
2. Bisabolol Oxide B
3. Alpha-Bisabolol
4. Alpha-Bisabolone Oxide
5. Chamazulene
6. Bisabolol Oxide A
7. Spiroether





Acknowledgement: Prof. C.Bicchi, C.Brunelli et al. Universita di Torino, Dipartimento di Scienza e Tecnologia del Farmaco, Via Pietro Giuria, 9, Torino, Italy

Conditions

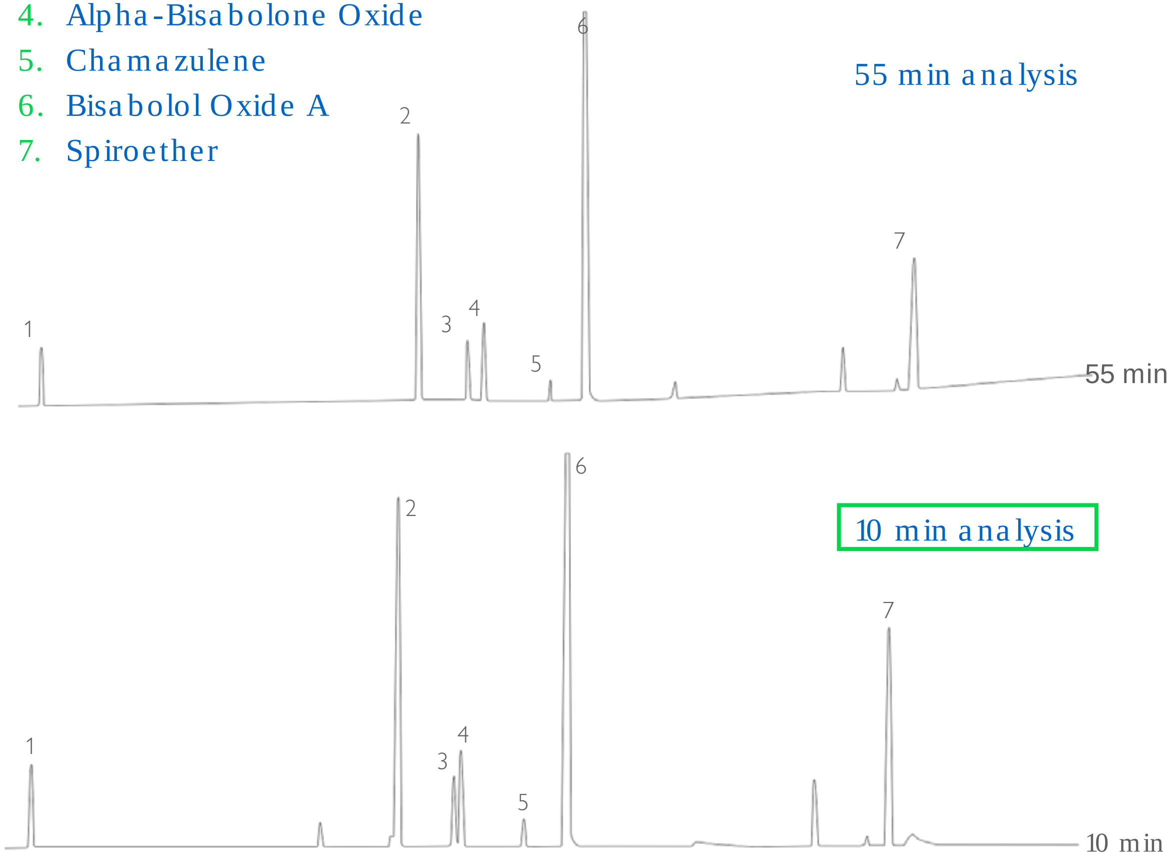
Standard GC Method

Oven Program:	50 °C (0.1 min), 3 °C/min, 250 °C (5 min)
Carrier Gas:	Hydrogen, 1.5 mL/min.
Injector:	Split 230 °C, 1 µL, 1:50 Split Ratio
Detector:	FID, 250 °C
Sample Dilution:	1% in Cyclohexane
Column details:	HI -1701, 0.25 mm, 0.30 µm, 25 m

avantor™

Comparison of Standard and FAST GC for Chamomile Analysis

1. Trans-Beta-Farnesene
2. Bisabolol Oxide B
3. Alpha-Bisabolol
4. Alpha-Bisabolone Oxide
5. Chamazulene
6. Bisabolol Oxide A
7. Spiroether



Acknowledgement: Prof. C.Bicchi, C.Brunelli et al. Universita di Torino, Dipartimento di Scienza e Tecnologia del Farmaco, Via Pietro Giuria, 9, Torino, Italy

Conditions

Standard GC Method

Oven Program:	50 °C (0.1 min), 3 °C/min, 250 °C (5 min)
Carrier Gas:	Hydrogen, 1.5 mL/min.
Injector:	Split 230 °C, 1 µL, 1:50 Split Ratio
Detector:	FID, 250 °C
Sample Dilution:	1% in Cyclohexane
Column details:	HI -1701, 0.25 mm, 0.30 µm, 25 m

Fast GC Method

Oven Program:	50 °C (0.1 min), 50 °C/min, 250 °C (5 min)
Carrier Gas:	Hydrogen, 0.5 mL/min.
Injector:	Split 230 °C, 0.5 µL, 1:250 Split Ratio
Detector:	FID, 250 °C
Sample Dilution:	1% in Cyclohexane
Column details:	HI-1701 FAST, 0.10 mm, 0.10 µm, 5 m

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Fast GC – How to select column dimensions

Use the Phase Ratio?

This is a value that characterizes film thickness and column internal diameter combinations and how retentive the combination is.

Lower β values result in increased retention.
Higher β values result in decreased retention.

Increasing retention →

Column diameter, d_c (mm)	Film thickness, d_f (µm)										
	0.15	0.18	0.25	0.5	1	1.4	1.5	1.8	2.65	3	5
0.15	250	208	150	75	38	27	25	21	14	13	8
0.18	300	250	180	90	45	32	30	25	17	15	9
0.25	417	347	250	125	63	45	42	35	24	21	13
0.32	533	444	320	160	80	57	53	44	30	27	16
0.53	883	736	530	265	133	95	88	74	50	44	27

Fast GC – Phase Ratio

<100 for highly volatile/low molecular weight analytes.
>400 for high molecular weight analytes

How is the Phase Ratio helpful?

Choose column with similar phase ratio when changing column dimensions, achieves similar retention.

Increasing retention →

Column diameter, d_c (mm)	Film thickness, d_f (µm)										
	0.15	0.18	0.25	0.5	1	1.4	1.5	1.8	2.65	3	5
0.15	250	208	150	75	38	27	25	21	14	13	8
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0.25	417	347	250	125	63	45	42	35	24	21	13
0.32	533	444	320	160	80	57	53	44	30	27	16
0.53	883	736	530	265	133	95	88	74	50	44	27

Fast GC – Phase Ratio

Example – What is the phase ratio of a 0.25 mm ID x 0.18 µm FT column?

$$\beta = \frac{d_c}{4d_f} \qquad \beta = \frac{250}{4 \times 0.18}$$

$$\beta = \frac{250}{0.72} \qquad \beta = 347.22 \checkmark$$

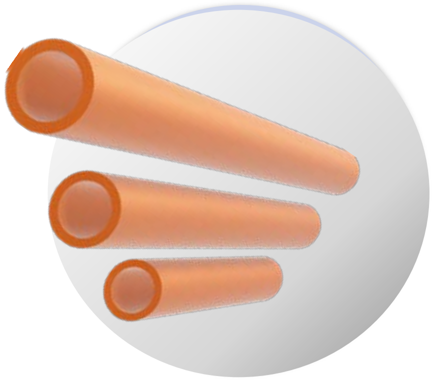
Phase Ratio formula -

$$\beta = \frac{d_c}{4d_f} \qquad \beta = \frac{\text{Column ID } (\mu m)}{4 \times \text{Film thickness } (\mu m)}$$

Increasing retention →

Column diameter, d_c (mm)	Film thickness, d_f (µm)										
	0.15	0.18	0.25	0.5	1	1.4	1.5	1.8	2.65	3	5
0.15	250	208	150	75	38	27	25	21	14	13	8
0.18	300	250	180	90	45	32	30	25	17	15	9
0.25	417	347	250	125	63	45	42	35	24	21	13
0.32	533	444	320	160	80	57	53	44	30	27	16
0.53	883	736	530	265	133	95	88	74	50	44	27

Fast GC method transfer summary



Smaller column dimensions and sample dilution

Faster oven ramp rate

Higher carrier gas linear velocity

To achieve Fast GC

Thank you for your attention

Any Questions?