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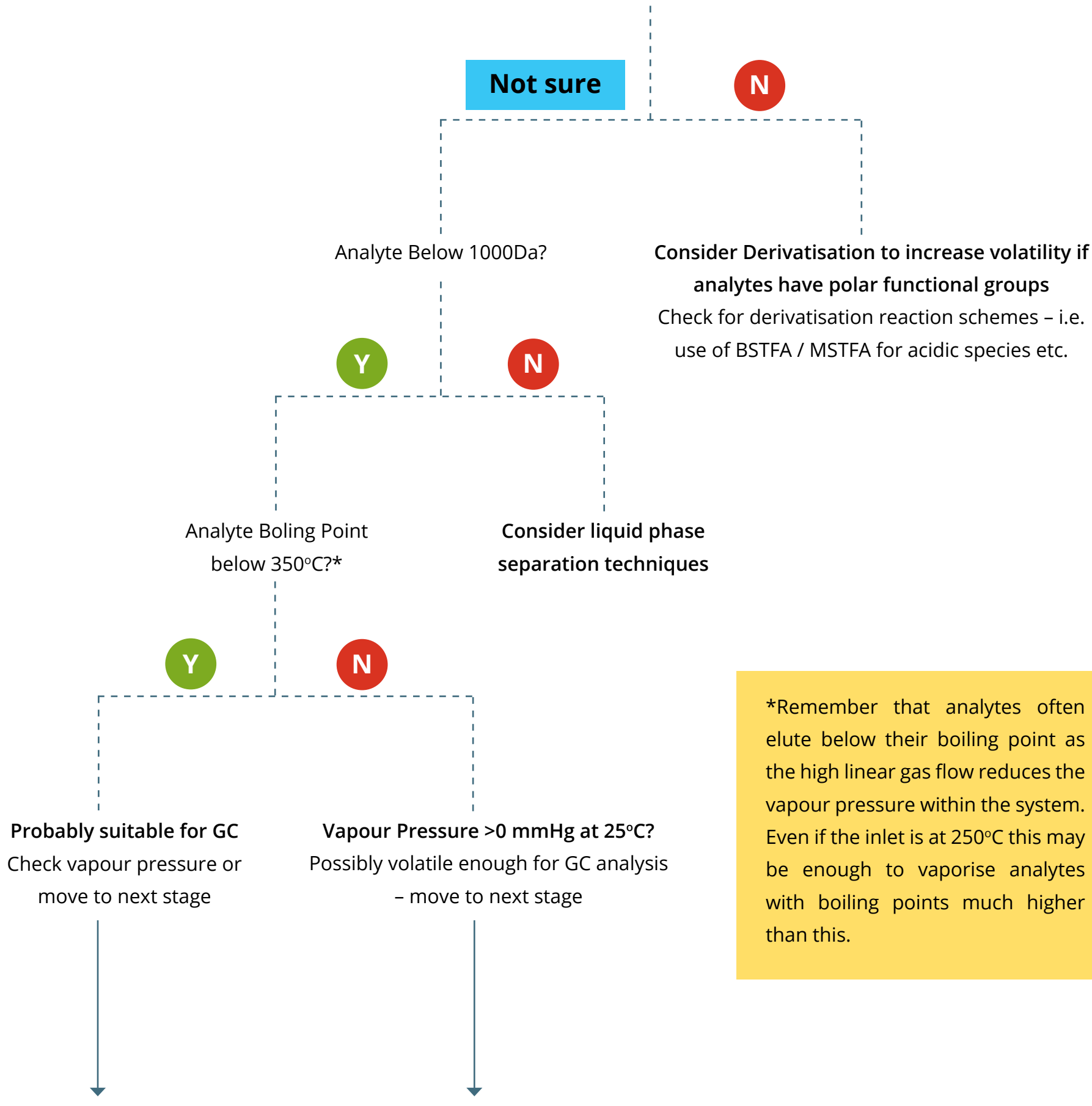
GC Method Development

A Decision Tree for Gas Chromatography Method Development

BUILD BETTER GC METHODS WITH CRAWFORD SCIENTIFIC

A DECISION TREE FOR GAS CHROMATOGRAPHY METHOD DEVELOPMENT

Stage 1 – Is analyte volatile enough for GC analysis?



*Remember that analytes often elute below their boiling point as the high linear gas flow reduces the vapour pressure within the system. Even if the inlet is at 250°C this may be enough to vaporise analytes with boiling points much higher than this.

A DECISION TREE FOR GAS CHROMATOGRAPHY METHOD DEVELOPMENT

Stage 2 – Nature of the sample and the requirement for sample preparation

Simple Sample (few components)

Solid

Choose appropriate sample solvent based on analyte (and matrix) polarity

Consider filtering the dissolved sample

Liquid

Choose appropriate sample solvent based on analyte (and matrix) polarity

Gas

Choose appropriate sampling and sample introduction technique

- Tedlar (gas sampling) bag
- Gas sampling valve
- Thermal desorption tube

If the final sample is in liquid form, consider boiling point and expansion co-efficient when optimising splitless injection conditions (where appropriate).

The final analyte concentration should be considered if analyte dilution is employed.

Move to next stage

Complex Sample (many components)

Solid

Choose appropriate sample solvent based on analyte (and matrix) polarity

Liquid

Consider The need for sample preparation in the following order of selectivity

- Solid Phase Extraction (SPE)
- Solid Phase Microextraction (SPME) / Stir Bar Sorbtive Extraction (SBSE)
- Dynamic Liquid / Liquid Extraction (DLLME)
- QuEChERS
- Liquid / Liquid Extraction (LLE)
- Filtration*

If the final sample is in liquid form, consider boiling point and expansion co-efficient when optimising splitless injection conditions (where appropriate).

The final analyte concentration should be considered if analyte dilution is employed.

Move to next stage

Gas

Choose appropriate sampling and sample introduction technique

- Tedlar (gas sampling) bag
- Gas sampling valve
- Thermal desorption tube

*Check the chemical compatibility of filter materials and housings when filtering organic solvents. Some assessment should also be made of the absorptivity of the filter material towards target analytes to ensure good accuracy and reproducibility.

A DECISION TREE FOR GAS CHROMATOGRAPHY METHOD DEVELOPMENT

Stage 3 (I) – Select appropriate detection technique: Quantitative Analysis

FID*

Default choice

- **Response Type:** *Universal (C)*
- **Responds To:** *Carbon*
- **Mass or Concentration Dependant:** *Mass*
- **Destructive:** *Y*
- **LOD:** *10^{-12} gC/sec*
- **Linear Range:** *10^{7}*
- **Relative Selectivity:** *none*
- **Uses:** *Default detector choice for organic compounds*
- **Optimise:** ***Temperature*** *(typically 300 – 350°C)* ***Air to hydrogen ratio*** *(typically 100:1)* ***Make-up gas flow*** *(match air flow and optimise in +/-20% steps)*

ECD

Analyte contains Oxygen / Halogens

- **Response Type:** *Selective*
- **Responds To:** *Halogen or Oxygen containing analytes*
- **Mass or Concentration Dependant:** *Concentration*
- **Destructive:** *N*
- **LOD:** *10^{-14} g/mL*
- **Linear Range:** *10^{4}*
- **Relative Selectivity:** *Up to 10^{6} versus carbon*
- **Uses:** *Environmental analysis for pesticides & herbicides*
- **Optimise:** ***Make up Gas Type & Flow*** *(Nitrogen, Methane or Methane with 5% Argon)* ***Temperature*** *(250 - 350°C typical)* ***Current & Mode*** *Magnitude of applied current / pulsed or constant current mode*

TCD

Permanent Gas Analysis

- **Response Type:** *Universal (C)*
- **Responds To:** *Thermal Conductivity*
- **Mass or Concentration Dependant:** *Concentration*
- **Destructive:** *N*
- **LOD:** *10^{-9} g/mL*
- **Linear Range:** *up to 10^{5}*
- **Relative Selectivity:** *none*
- **Uses:** *Permanent gas analysis or where non-destructive general detector is required*
- **Optimise:** ***Temperature*** *(250 - 350°C typical)* ***Bead Power / Voltage*** *(follow manufacturers guidelines)* ***Hydorgen Flow*** *(low enough not avoid a flame formation)*

NPD

Analyte contains N / P

- **Response Type:** *Universal (C)*
- **Responds To:** *Nitrogen or Phosphorous*
- **Mass or Concentration Dependant:** *Mass*
- **Destructive:** *Y*
- **LOD:** *10^{12} gN/sec*
- **Linear Range:** *10^{5}*
- **Relative Selectivity:** *2 x 10^{4} v C (N)* *7 x 10^{4} v C (P)*
- **Uses:** *Environmental analysis / Petrochemical analysis*
- **Optimise:** ***Temperature*** *(lowest works best)* ***Reference flow*** *(typically 3x column + makeup flow)* ***Filament resistance*** *(follow manufacturer guidelines)*

FPD

Analyte contains S / P

- **Response Type:** *Universal (C)*
- **Responds To:** *Phosphorous or Sulphur*
- **Mass or Concentration Dependant:** *Mass*
- **Destructive:** *Y*
- **LOD:** *10^{-13} gP/sec* *10^{-12} gS/sec*
- **Linear Range:** *10^{4} (P)* *Non-Linear (S)*
- **Relative Selectivity:** *10^{6} v C (P)* *10^{6} v C (S)*
- **Uses:** *Any analysis requiring trace determination of P or S containing compounds*
- **Optimise:** ***Temperature*** *(up to 250°C typical)* ***Filter*** *(select correct filter- either P or S)* ***As per FID***

CLD

Analyte contains S / N

- **Response Type:** *Universal (C)*
- **Responds To:** *Nitrogen or Sulphur*
- **Mass or Concentration Dependant:** *Mass*
- **Destructive:** *Y*
- **LOD:** *10^{-12} gS/sec* *10^{-12} gN/sec*
- **Linear Range:** *>10^{4} (S & N)*
- **Relative Selectivity:** *10^{7} v C (S)* *10^{7} v C (N)*
- **Uses:** *Petroleum characterisation, flavour analysis, environmental monitoring*
- **Optimise:** ***Temperature Furnace Temperature*** *(800°C is typical)* ***Ozone Flow Hydrogen Flow Air Flow*** *(follow manufacturer guidelines)*

Move to next stage

*FID – Flame Ionisation Detector / ECD – Electron Capture Detector / TCD – Thermal Conductivity Detector / NPD – Nitrogen Phosphorous Detector / FPD – Flame Photometric Detector / CLD – Chemiluminesence Detector. Also available – Photoionisation detector (PID) / Atomic Emission Detector (AED) / Electrolytic Conductivity Detector (ELCD) / Infrared Detector (IRD)

Stage 3 (II) – Select appropriate detection technique: Qualitative and / or Quantitative Analysis

Mass Spectrometric Detection
Electron Ionisation (EI) Mode

- **Response Type:**
  Selective & Universal
- **Responds To:**
  Radical Cations created in ion source
- **Mass or Concentration Dependant:**
  Mass
- **Destructive:**
  Y
- **LOD:**
  10^{6}
- **Linear Range:**
  10^{7}
- **Relative Selectivity:**
  0.1Da in selected ion or higher in single or multiple reaction monitoring (SRM / MRM) modes
- **Uses:**
  Universal for qualitative analysis
- **Optimise:**
  Temperature of Transfer Line, Ion Source and Mass Analyser
  The MSD is a complex detector which requires both calibration and optimisation using onboard tuning algorithms

Poor Molecular Ion Formation or
Sensitivity in EI mode

Try EI at 25eV electron energy to reduce degree of fragmentation and promote intensity of molecular ion

Mass Spectrometric Detection
Chemical Ionisation (CI) Mode

+ve

- **Chose Reagent Gas:**
  Isobutane
  Methane
  Ammonia
  (in order of decreasing molecular ion intensity)
- **LOD:**
  10^{-6} g

-ve

- **Chose Reagent Gas:**
  Isobutane
  Methane
  Ammonia
  (in order of decreasing molecular ion intensity)
- **LOD:**
  10^{-15} g

Move to next stage

A DECISION TREE FOR GAS CHROMATOGRAPHY METHOD DEVELOPMENT

Stage 4 – Select appropriate sample introduction technique[1,2]

Split / Splitless Injection

Default choice for volatile / non-labile samples

Split Injection
Optimise:
Liner Type
(Straight through / deactivated / no packing / no restriction is typical)
Inlet Temperature
(250°C is typical – optimise in steps of +/-50°C checking %RSD of repeat injections and speak shape)
Injection Volume
(1µL is typical – calculate sample expansion volume doesn't exceed liner volume)3
Split Ratio
(100:1 is typical – optimise in +/- 20:1 steps

Spitless Injection
Optimise:
Liner Type
(Packed / deactivated / bottom restriction is typical)
Injection Volume
(1µL is typical – calculate sample expansion volume doesn't exceed liner volume)
Splitless Time
(based on sample transfer time from inlet to column)
Initial Oven Isothermal Temperature
(should be 20°C lower than sample diluent boiling point)
Initial Oven Isothermal Time
(at least as long as the Splitless time)

Programmed Thermal Vaporising (Large Volume) Injection

Thermally labile analytes or ultra-trace analysis

Optimise:
Mode
(Solvent Vent / Cold Split / Cold Splitless)

Initial Inlet Temperature

Initial Inlet Time

Vent flow pressure

Vent Flow Time

Purge Flow

Purge Time

Injection Volume

Injection Speed
Cryogenic Cooling Time
Oven Temperature Tracking

Static Headspace Sampling

Volatile analytes in involatile liquids or solids

Optimise:
Sample to headspace ratio
(Determined by the sample volume to headspace vial size)
Equilibration Temp.
Equilibration Time
Shaking / Agitation On/ Off & Speed
Sample loop size
(1mL or 3mL loops are typical)
Vial Equilibration Pressure and Time

Loop Filling Temperature and Time

Relative temperature of sample transfer line and GC inlet

Split flow to promote efficient gas plug transfer

Initial GC oven temperature or cryo-focussing time

Purge & Trap Sampling

For increased selectivity and analyte preconcentration (liquid or solid samples)

Optimise:
Purge Temperature

Purge Flow

Purge Volume

Trap Sorbent Material Chemistry

Desorb Pre-heat

Desorb Time

Dry Purge Temperature / Time / Flow
(Sometimes required to reduce carry-over between samples)

Sample size and purge solvent volume for solid samples

Thermal Desorption Sampling

For increased selectivity and analyte preconcentration (gaseous samples)

Optimise:
Trap Sorbent Material Chemistry

Desorb Temperature

Desorb Flow Rate

Inlet Split Ratio

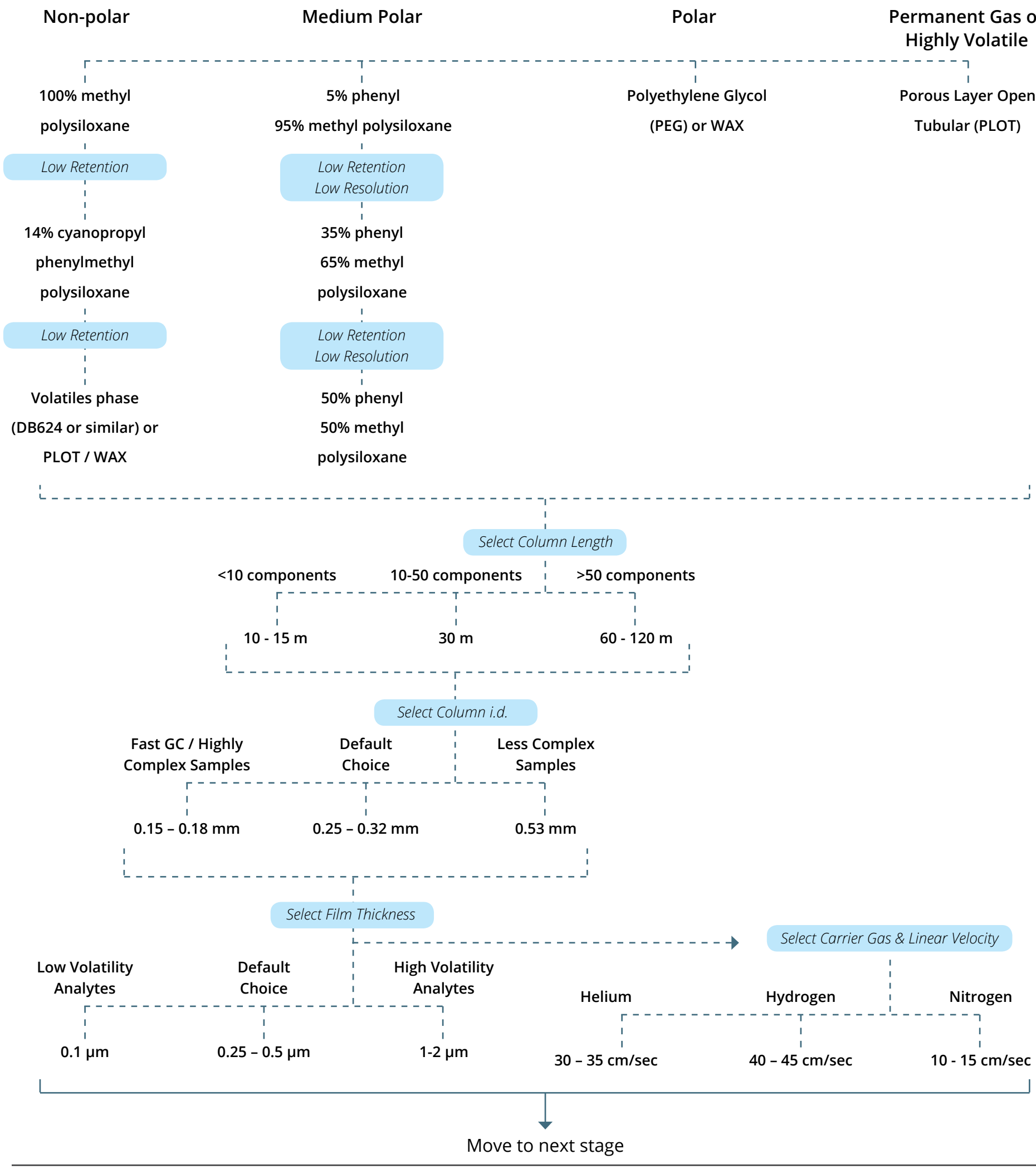
Dry Purge Temperature / Time / Flow

1. In each case we have highlighted the broad principles behind optimisation of these techniques. For more in-depth information visit the relevant topic on www.CHROMacademy.com
2. Whilst we have proposed the parameters to be optimised in what we believe to be priority order, some iteration may be required to find the optimum parameter set
3. For more information on calculators for vapour volume and pressure flow relationships – please visit; https://www.agilent.com/en/support/gas-chromatography/gccalculators https://www.restek.com/Technical-Resources/Chromatography-Calculators or several of the other freely available calculators from GC column manufacturers

Move to next stage

A DECISION TREE FOR GAS CHROMATOGRAPHY METHOD DEVELOPMENT

Stage 5 – Select appropriate GC Column



A DECISION TREE FOR GAS CHROMATOGRAPHY METHOD DEVELOPMENT

Stage 6 – Develop & Optimise Oven Temperature Program [1,2]

Splitless Injection

Set Initial Gradient Temperature & Hold Time

Follow screen method for Split injection but insert initial hold time of 1 min.

Maintain Initial Temperature at 20°C below boiling point of sample diluent

Optimise hold time empirically in steps of +/- 10 seconds until peak area of early eluting peaks is <1% RSD

Follow Split Temperature Program Optimisation

Split Injection

Screen sample using the following conditions

Initial Temp.:	40°C
Initial Time:	0 mins.
Programming Rate:	10°C/min.
Final Temp.:	330°C
	(or column gradient max. temp.)
Final Time:	10 mins

Peaks elute in a window of 5 mins or less?

Y

Isothermal Analysis Possible

Calculate $T_{(f)}$ (retention temperature of final peak in the screening chromatogram)

$$T_{iso} = T_{(f)} - 45°C$$

N

Set Initial Gradient Temperature

Calculate $T_{(i)}$ (retention temperature of first analyte peak in the screening chromatogram)

$$T_{initial} = T_{(i)} - 45°C$$

Optimise Temperature Ramp Rate

Calculate or measure t_0 (hold-up time)
Optimum Ramp Rate = 10°C per t0
Further optimise in steps of +/- 10°C/min.

Optimise Final Temperature & Time

Final temperature is
$T_{(final)} = T_{(f)} + 20°C$
Final Hold Time is t_0 x 5 mins (optimise empirically)

Optimise resolution for critical pairs using mid-ramp holds

Determine elution temperature of critical pair $T_{(crit)}$ and insert mid ramp hold at
$T_{(iso\text{-}hold)} = T_{(crit)} - 45°C$

Optimise the mid-ramp hold time empirically

1. The various relationships shown here are 'rules' of thumb' and need to be used as such to guide next steps. There are more finessed calculations which can be found at www.CHROMacademy.com

2. It is likely that some empirical optimisation will be required after each rule of thumb is employed and the results of the calculations will not deliver the optimum possible value for that variable.

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ABOUT THE AUTHOR

Tony Taylor has over 30 years of experience in developing chromatographic methods. As the Technical Director of three varied contract and application development laboratories, he understands what frustrates analytical chemists and how to help them overcome problems.
He has helped thousands of budding chromatography method developers using his own experiences and insights, working with students to improve knowledge and understanding of chromatographic processes and their application.