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?

- Not sure
  - Analyte Below 1000Da?
    - Y
      - Analyte boiling point below 350°C?*
        - Y
          - Probably suitable for GC
            - Check vapour pressure or move to next stage
        - N
          - Vapour Pressure >0 mmHg at 25°C?
            - Y
              - Possibly volatile enough for GC analysis – move to next stage
            - N
              - Consider derivatisation to increase volatility if analytes have polar functional groups
                - Check for derivatisation reaction schemes – i.e. use of BSTFA / MSTFA for acidic species etc.

- N
  - Consider liquid phase separation techniques

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

Simple Sample (few components) | Complex Sample (many components)
--- | ---
Solid | Solid
Choose appropriate sample solvent based on analyte (and matrix) polarity
Consider filtering the dissolved sample

Liquid | Liquid
Choose appropriate sample solvent based on analyte (and matrix) polarity
Choose appropriate sampling and sample introduction technique
• Tedlar (gas sampling) bag
• Gas sampling valve
• Thermal desorption tube

Gas | Gas
Choose appropriate sample solvent based on analyte (and matrix) polarity
Consider The need for sample preparation in the following order of selectivity
• Solid Phase Extraction (SPE)
• Solid Phase Microextraction (SPME) / Stir Bar Sorptive 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 coefficient when optimising splitless injection conditions (where appropriate).

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

Move to next stage

*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

<table>
<thead>
<tr>
<th>FID*</th>
<th>ECD</th>
<th>TCD</th>
<th>NPD</th>
<th>FPD</th>
<th>CLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Default choice</td>
<td>Analyte contains Oxygen / Halogens</td>
<td>Permanent Gas Analysis</td>
<td>Analyte contains N / P</td>
<td>Analyte contains S / P</td>
<td>Analyte contains S / N</td>
</tr>
<tr>
<td>• Response Type: Universal (C)</td>
<td>• Response Type: Selective</td>
<td>• Response Type: Universal (C)</td>
<td>• Response Type: Universal (C)</td>
<td>• Response Type: Universal (C)</td>
<td>• Response Type: Universal (C)</td>
</tr>
<tr>
<td>• Responds To: Carbon</td>
<td>• Responds To: Halogen or Oxygen containing analytes</td>
<td>• Responds To: Thermal Conductivity</td>
<td>• Responds To: Nitrogen or Phosphorous</td>
<td>• Responds To: Phosphorous or Sulphur</td>
<td>• Responds To: Nitrogen or Sulphur</td>
</tr>
<tr>
<td>• Mass or Concentration Dependant: Mass</td>
<td>• Mass or Concentration Dependant: Concentration</td>
<td>• Mass or Concentration Dependant: Mass</td>
<td>• Mass or Concentration Dependant: Mass</td>
<td>• Mass or Concentration Dependant: Mass</td>
<td>• Mass or Concentration Dependant: Mass</td>
</tr>
<tr>
<td>• Destructive: N</td>
<td>• Destructive: N</td>
<td>• Destructive: N</td>
<td>• Destructive: N</td>
<td>• Destructive: N</td>
<td>• Destructive: N</td>
</tr>
<tr>
<td>• LOD: $10^{-12}$ gC/sec</td>
<td>• LOD: $10^{-14}$ g/mL</td>
<td>• LOD: $10^{-9}$ g/mL</td>
<td>• LOD: $10^{-12}$ gS/sec</td>
<td>• LOD: $10^{-12}$ gS/sec</td>
<td>• LOD: $10^{-12}$ gS/sec</td>
</tr>
<tr>
<td>• Linear Range: $10^7$</td>
<td>• Linear Range: $10^6$</td>
<td>• Linear Range: $10^7$</td>
<td>• Linear Range: $10^7$</td>
<td>• Linear Range: $10^7$</td>
<td>• Linear Range: $10^7$</td>
</tr>
<tr>
<td>• Relative Selectivity: none</td>
<td>• Relative Selectivity: none</td>
<td>• Relative Selectivity: none</td>
<td>• Relative Selectivity: none</td>
<td>• Relative Selectivity: none</td>
<td>• Relative Selectivity: none</td>
</tr>
<tr>
<td>• Uses: Environmental analysis for pesticides &amp; herbicides</td>
<td>• Uses: Environmental analysis or where non-destructive general detector is required</td>
<td>• Uses: Environmental analysis / Petrochemical analysis</td>
<td>• Uses: Any analysis requiring trace determination of P or S containing compounds</td>
<td>• Uses: Petroleum characterisation, flavour analysis, environmental monitoring</td>
<td>• Uses: Petroleum characterisation, flavour analysis, environmental monitoring</td>
</tr>
<tr>
<td>• 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)</td>
<td>• Optimise: Make up Gas Type &amp; Flow (Nitrogen, Methane or Methane with 5% Argon) Temperature (250 – 350°C typical) Current &amp; Mode Magnitude of applied current / pulsed or constant current mode</td>
<td>• Optimise: Temperature (250 - 350°C typical) Bead Power / Voltage (follow manufacturers guidelines) Hydrogen Flow (low enough not avoid a flame formation)</td>
<td>• Optimise: Temperature (lowest works best) Reference flow (typically 3x column + makeup flow) Filament resistance (follow manufacturer guidelines)</td>
<td>• Optimise: Temperature (up to 250°C typical) Filter (select correct filter - either P or S) As per FID</td>
<td>• Optimise: Temperature Furnace Temperature (800°C is typical) Ozone Flow Hydrogen Flow Air Flow (follow manufacturer guidelines)</td>
</tr>
</tbody>
</table>

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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

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

- **Mass Spectrometric Detection**
  - Chemical Ionisation (CI) Mode

- **Chose Reagent Gas:**
  - Isobutane
  - Methane
  - Ammonia
  - (in order of decreasing molecular ion intensity)

- **LOD:**
  - $10^{-6}$ g

- **Chose Reagent Gas:**
  - Isobutane
  - Methane
  - Ammonia
  - (in order of decreasing molecular ion intensity)

- **LOD:**
  - $10^{-15}$ g

Move to next stage
Stage 4 – Select appropriate sample introduction technique\textsuperscript{1,2}

### Split / Splitless Injection

**Default choice for volatile / non-labile samples**

**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 peak shape)
- **Injection Volume**
  - (1μL is typical – calculate sample expansion volume doesn’t exceed liner volume)\textsuperscript{3}
- **Split Ratio**
  - (100:1 is typical – optimise in +/- 20:1 steps)

**Split Injection**

**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**

**Splitless 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:**
- **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**

### Static Headspace Sampling

**Volatile analytes in involatile liquids or solids**

**Optimise:**
- **Purge Temperature**
- **Purge Flow**
- **Purge Volume**
- **Trap Sorbent Material Chemistry**
- **Desorb Pre-heat**
- **Desorb Time**
- **Dry Purge Temperature / Time / Flow**

### Purge & Trap Sampling

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

**Optimise:**
- **Trap Sorbent Material Chemistry**
- **Desorb Temperature**
- **Desorb Flow Rate**
- **Inlet Split Ratio**
- **Dry Purge Temperature / Time / Flow**

### 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

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Move to next stage
Stage 5 – Select appropriate GC Column

Non-polar

100% methyl polysiloxane
- Low Retention

14% cyanopropyl phenylmethyl polysiloxane
- Low Retention

Volatile phase (DB624 or similar) or PLOT / WAX

Medium Polar

5% phenyl 95% methyl polysiloxane
- Low Retention
- Low Resolution

35% phenyl 65% methyl polysiloxane
- Low Retention
- Low Resolution

Polar

Polyethylene Glycol (PEG) or WAX
- Low Retention

50% phenyl 50% methyl polysiloxane

Permanent Gas or Highly Volatile

Porous Layer Open Tubular (PLOT)

Select Column Length

<10 components 10-50 components >50 components
- 10 - 15 m 30 m 60 - 120 m

Select Column i.d.

Fast GC / Highly Complex Samples Default Choice Less Complex Samples
- 0.15 – 0.18 mm 0.25 – 0.32 mm 0.53 mm

Select Film Thickness

Low Volatility Analytes Default Choice High Volatility Analytes
- 0.1 μm 0.25 – 0.5 μm 1-2 μm

Select Carrier Gas & Linear Velocity

Helium 30 – 35 cm/sec
Hydrogen 40 – 45 cm/sec
Nitrogen 10 - 15 cm/sec

Move to next stage
Stage 6 – Develop & Optimise Oven Temperature Program 1,2

**Splitless Injection**

1. Set Initial Gradient Temperature & Hold Time
2. Follow screen method for Split injection but insert initial hold time of 1 min.
3. Maintain Initial Temperature at 20°C below boiling point of sample diluent
4. Optimise hold time empirically in steps of +/- 10 seconds until peak area of early eluting peaks is <1% RSD

**Split Injection**

1. 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 $T_{iso}$ (retention temperature of final peak in the screening chromatogram)
  - $T_{iso} = T_{(f)} - 45°C$
  - Calculate $T_{initial}$ (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 $t_0$
  - 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 $t_0 \times 5$ 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 $T_{(iso-hold)} = T_{(crit)} - 45°C$
  - Optimise the mid-ramp hold time empirically

- **N**: Follow Split Temperature Program Optimisation

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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.
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.