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氣相層析儀的技術原理及其在生命科學研究的應用

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大綱

- 氣相層析原理
- 實際應用

層析技術的定義?

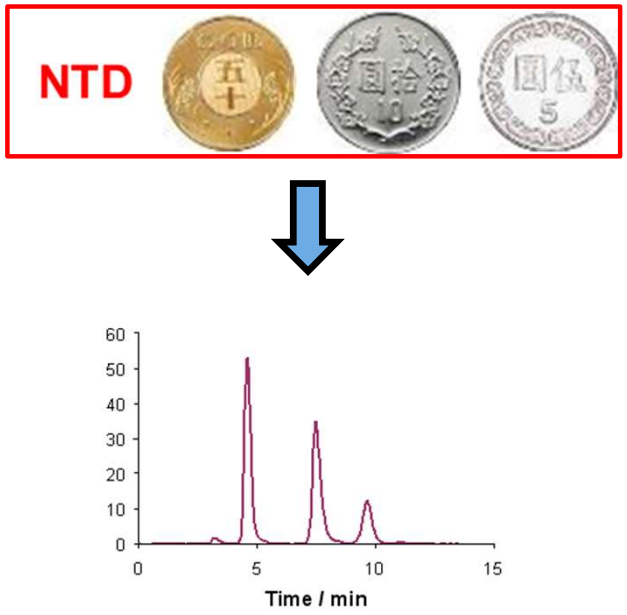
“一種將混合物以溶液或懸浮液通過組分以不同速率移動的介質來分離混合物的技術.”

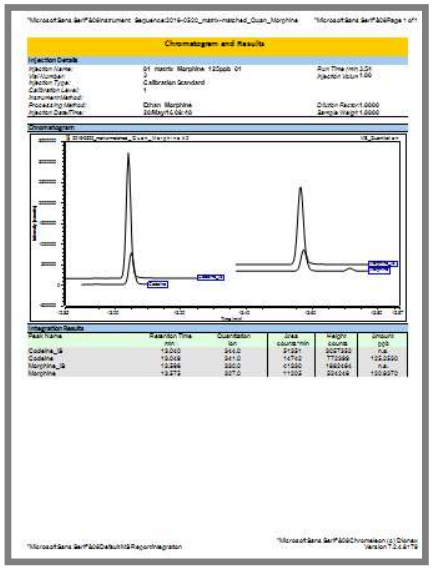
1. 氣相層析是一種用於分離揮發性有機化合物的技術.
2. 氣相層析儀由流動相、進樣口、包含固定相的分離柱、檢測器和數據記錄系統組成.
3. 由於色譜柱(管柱)中流動氣相和固定相之間的分配行為不同，來將有機化合物被分離.

儀器分析

樣品前處理 → 儀器分析 → 數據處理

Source: from Google





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如何開始分析?

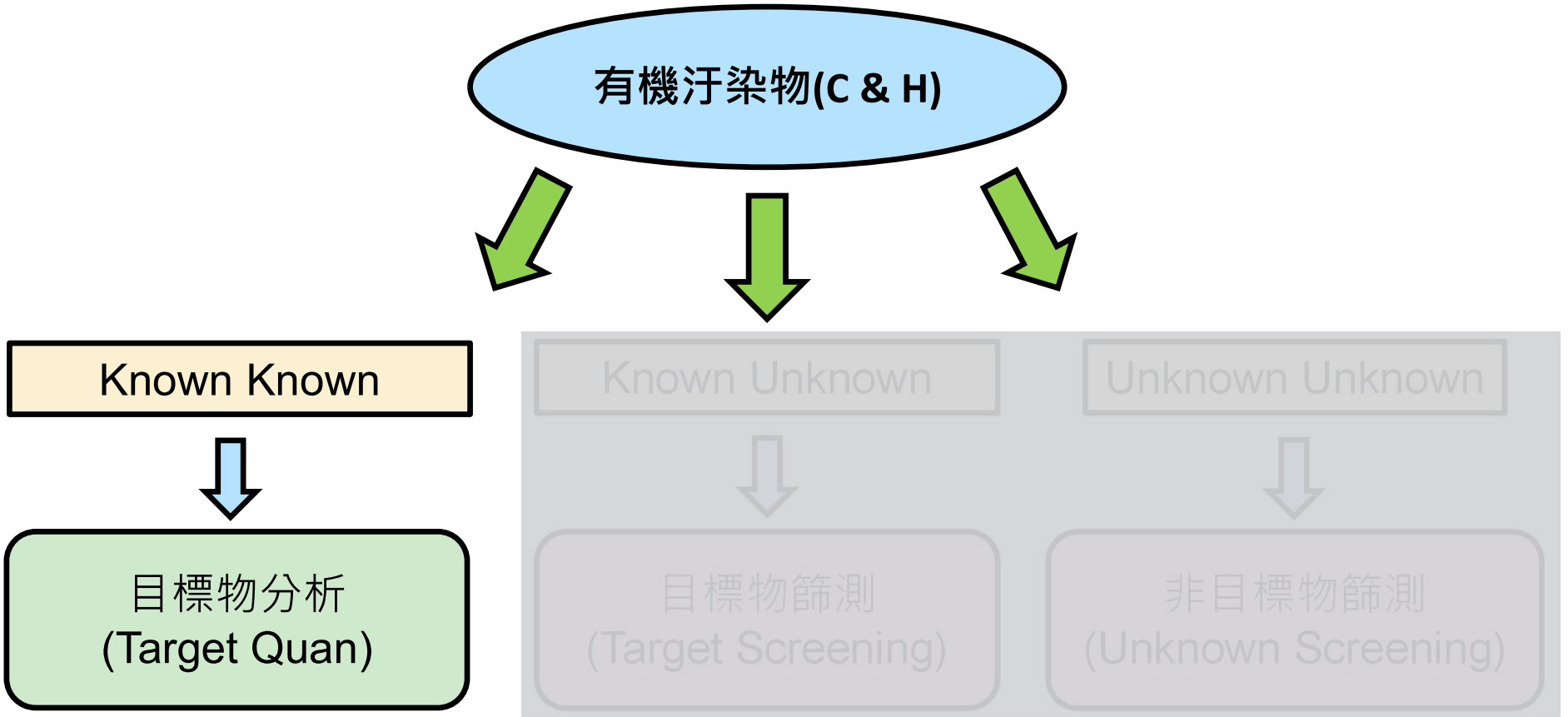
- 樣品的選擇?
- 是極性/非極性嗎？揮發性的？熱穩定?
- 我的樣品是濃縮的嗎？(濃度範圍).
- 您的方法目標?
- 溶劑是甚麼?
- 樣品注入量?
- 樣品中預計有哪些?

Liquid

Gas

Solid

有機分析/類別

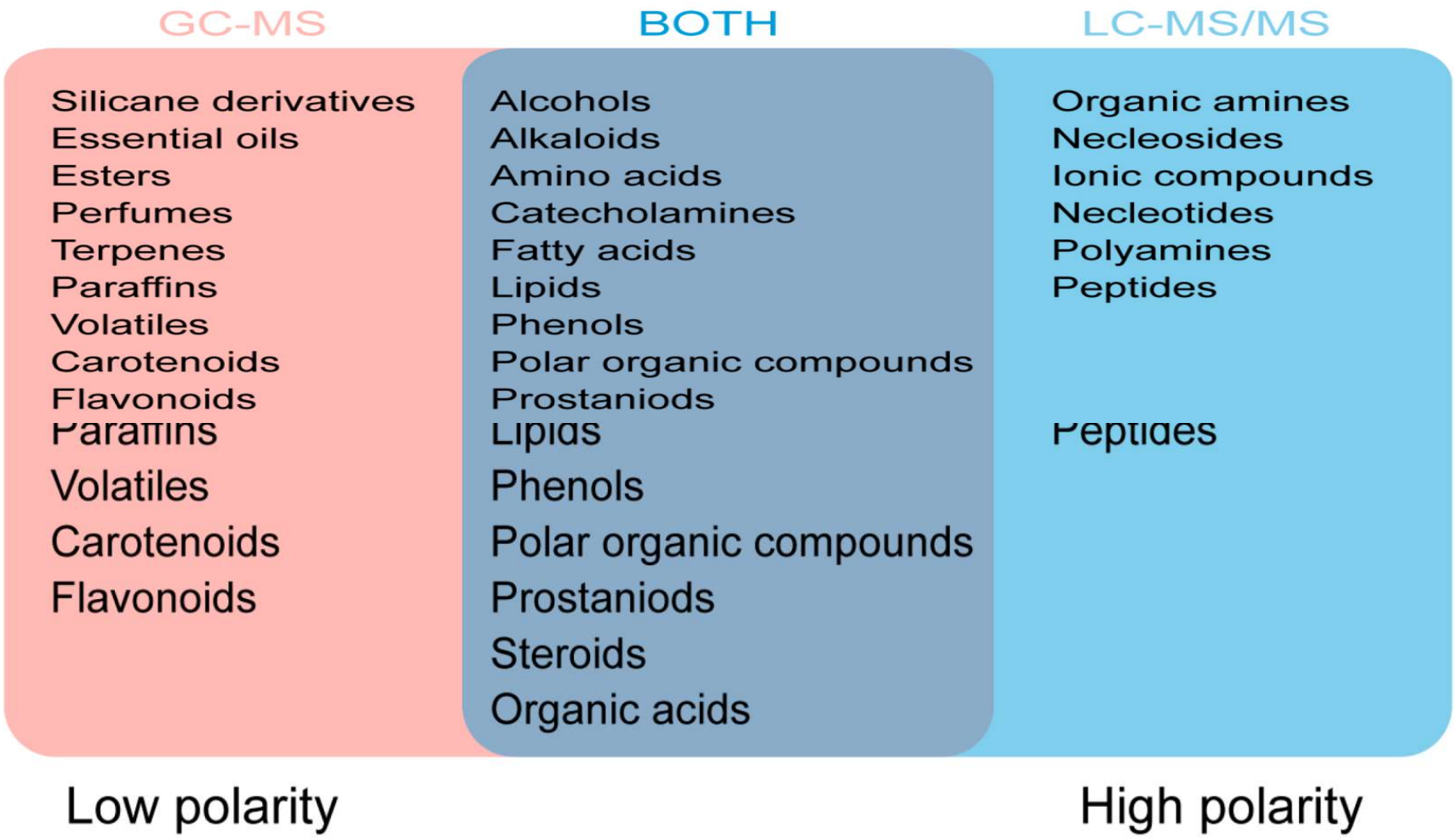


層析技術種類

For Gas Phase Sample (Volatile Sample)	→	Gas Chromatography (GC-TCD, GC-FID)
For Liquid Phase Sample (Non-Volatile Sample)	→	Liquid Chromatography (LC-UV, LC-DAD)
For Ionic Contaminants	→	Ion Chromatography (IC-RI, IC-CAD)
For Metal Components	→	Inductive Coupling Plasma (ICP-OES)
⋮		

GC或LC ??

- GC: 低分子量；氣體；有機溶劑
- LC: 高分子量；蛋白質；胜肽；核苷酸；藥物 (半揮發性、非揮發性、熱不穩定)



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TRACE 1300 Series GC – 1300 / 1310

TRACE 1310 GC:

觸控式螢幕介面，提供方便且簡單的儀器控制功能

TRACE 1300 GC:

本機內置的超簡化的用戶界面

- 2 buttons & 4 LEDs

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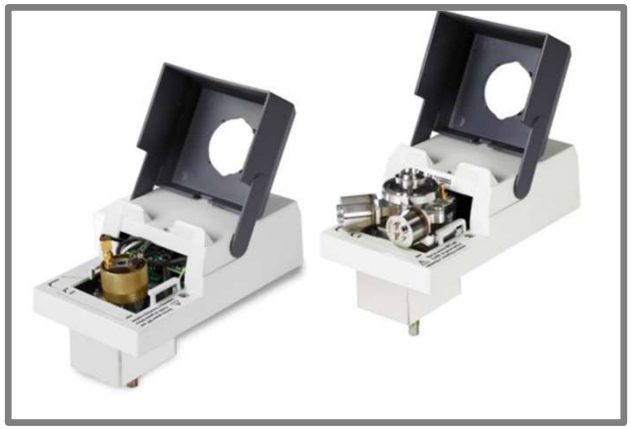
模組化GC / “即時連接 (instant connect)”

拆裝只需3個螺絲



注射口與偵測器模組
- 升級容易
- 因應未來需求

維護方便、簡單!!

Trace 1310 GC

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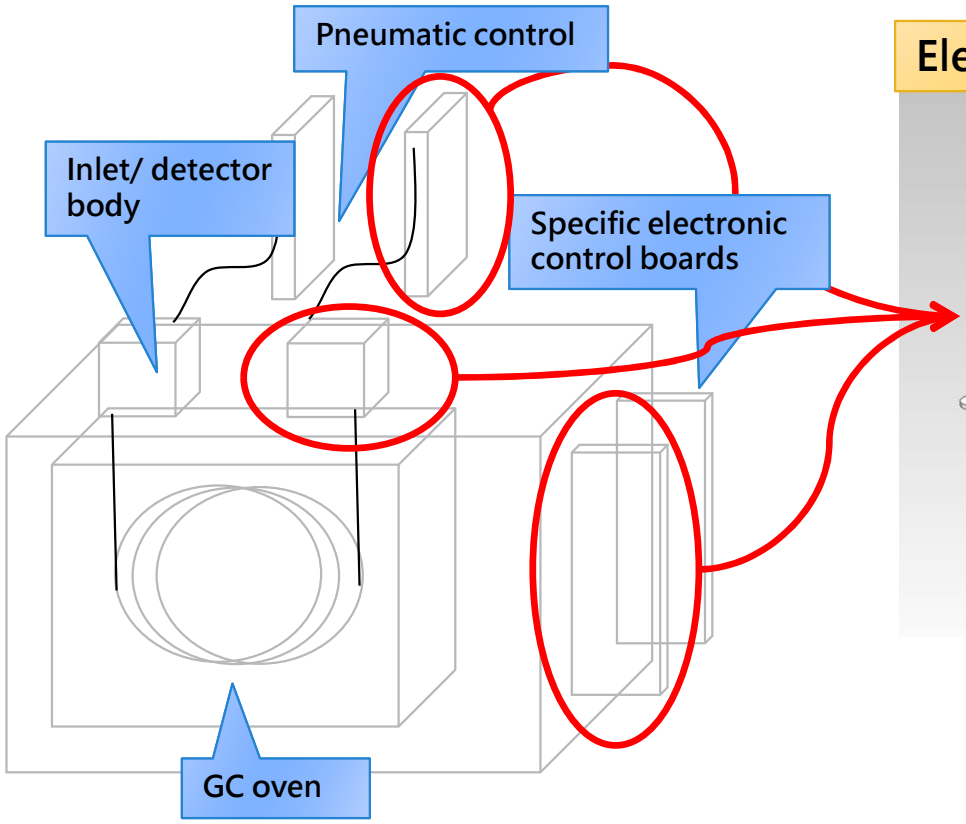
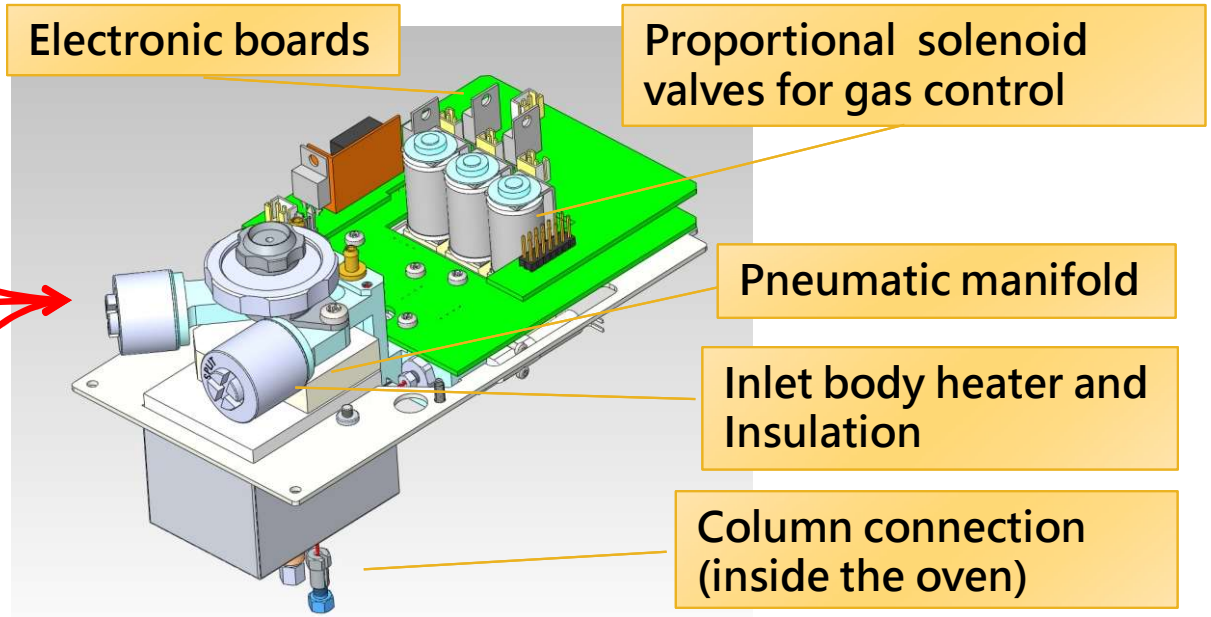
全新的儀器模組化設計





- **即時連接模組包括：**
  - 注射器和偵測器本體和加熱器
  - 氣體控制閥
  - 電子式溫度計、氣體流量控制器、訊號放大器、A/D轉換

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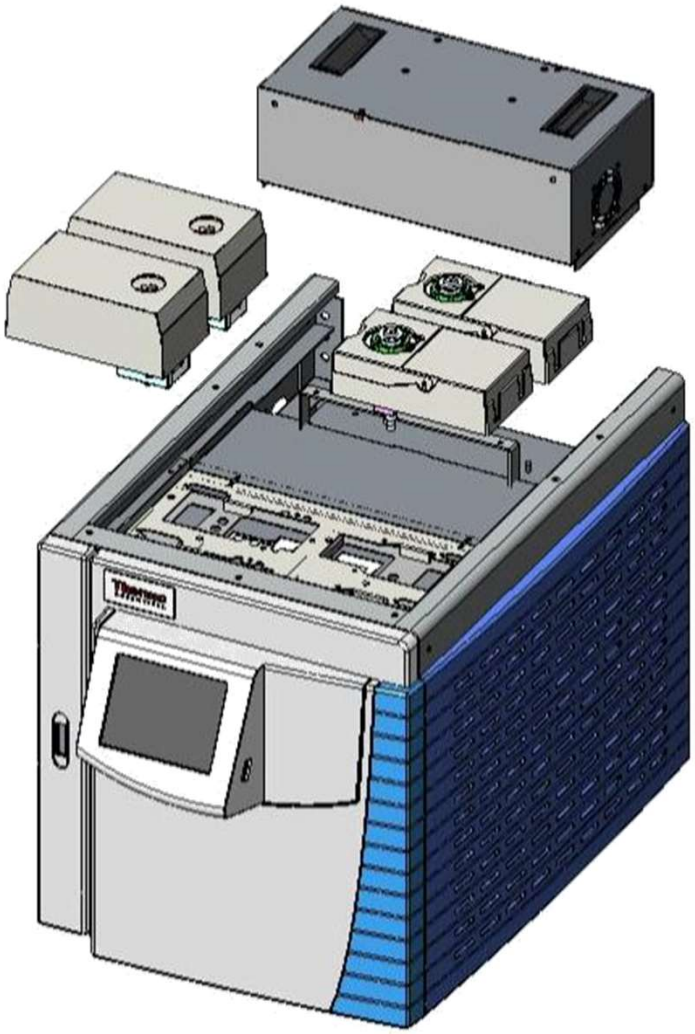
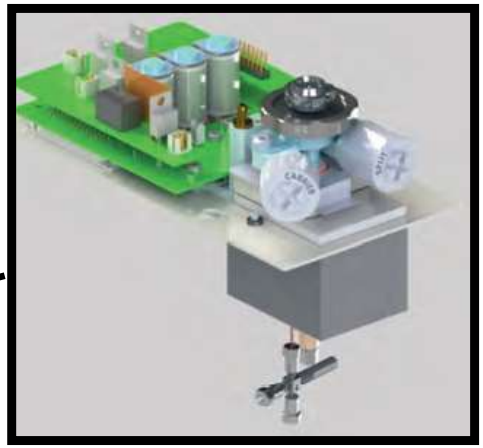
全新的儀器模組化設計

- **無須固定配置，按應用需求隨時更換配備**

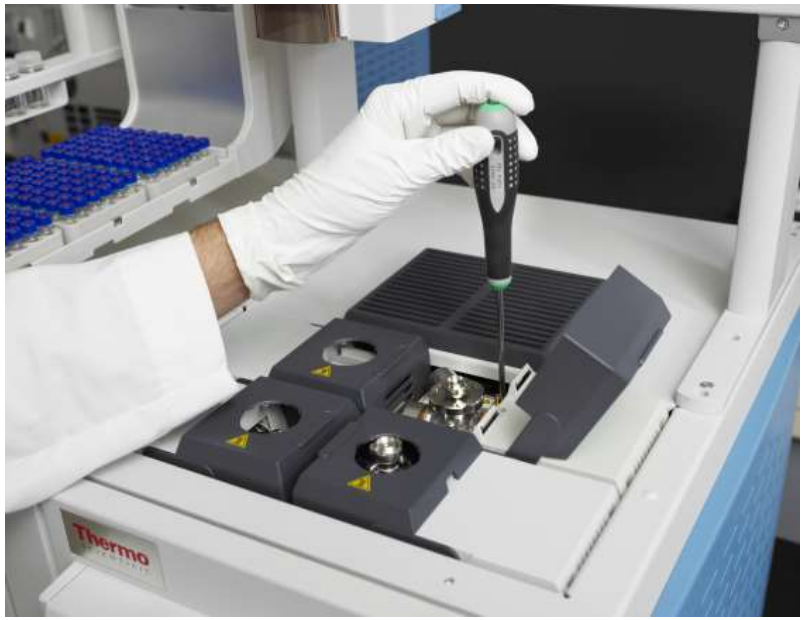
無多餘管線的模組化設計!!



SSL



FID



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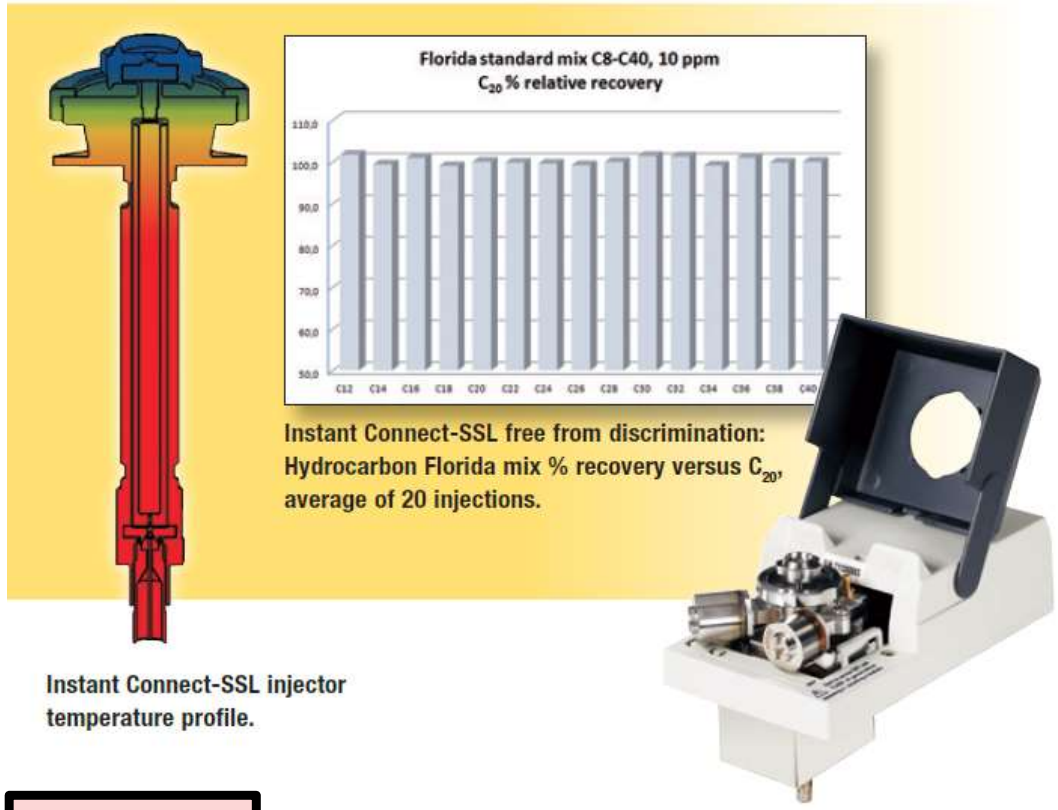
模組化GC / 注射口

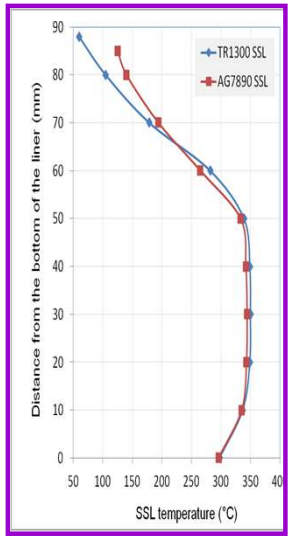
- SSL (Split/Splitless, 分流不分流); SSLBKF
- PTV (Programmable temperature vaporizer, 可變溫注射口); PTVBKF
- GSV (Gas sampling valve, 氣體進樣閥)



SSL

- 通用型 (Universal)
- 沒有歧視效應 (Free from discrimination)
- IEC裝置提高分析再現性 (Repeatability)
- 模組化，保養容易
- **換墊片 (septa) 不須降溫**

IEC: Integrated Electronic Control



注射口溫度分布

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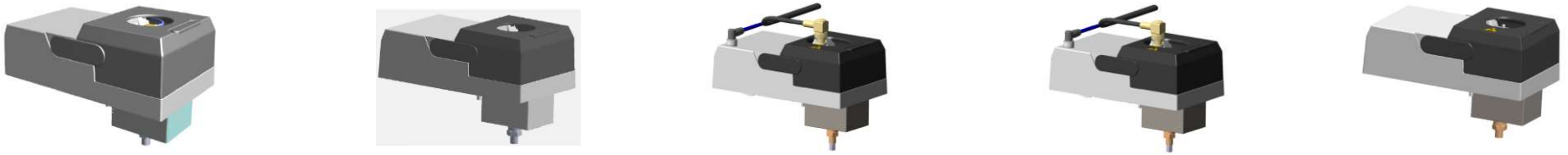
GC偵測器總覽

FID ECD NPD FPD TCD



- ISQ
- TSQ8000EVO
- Orbitrap

MS GC

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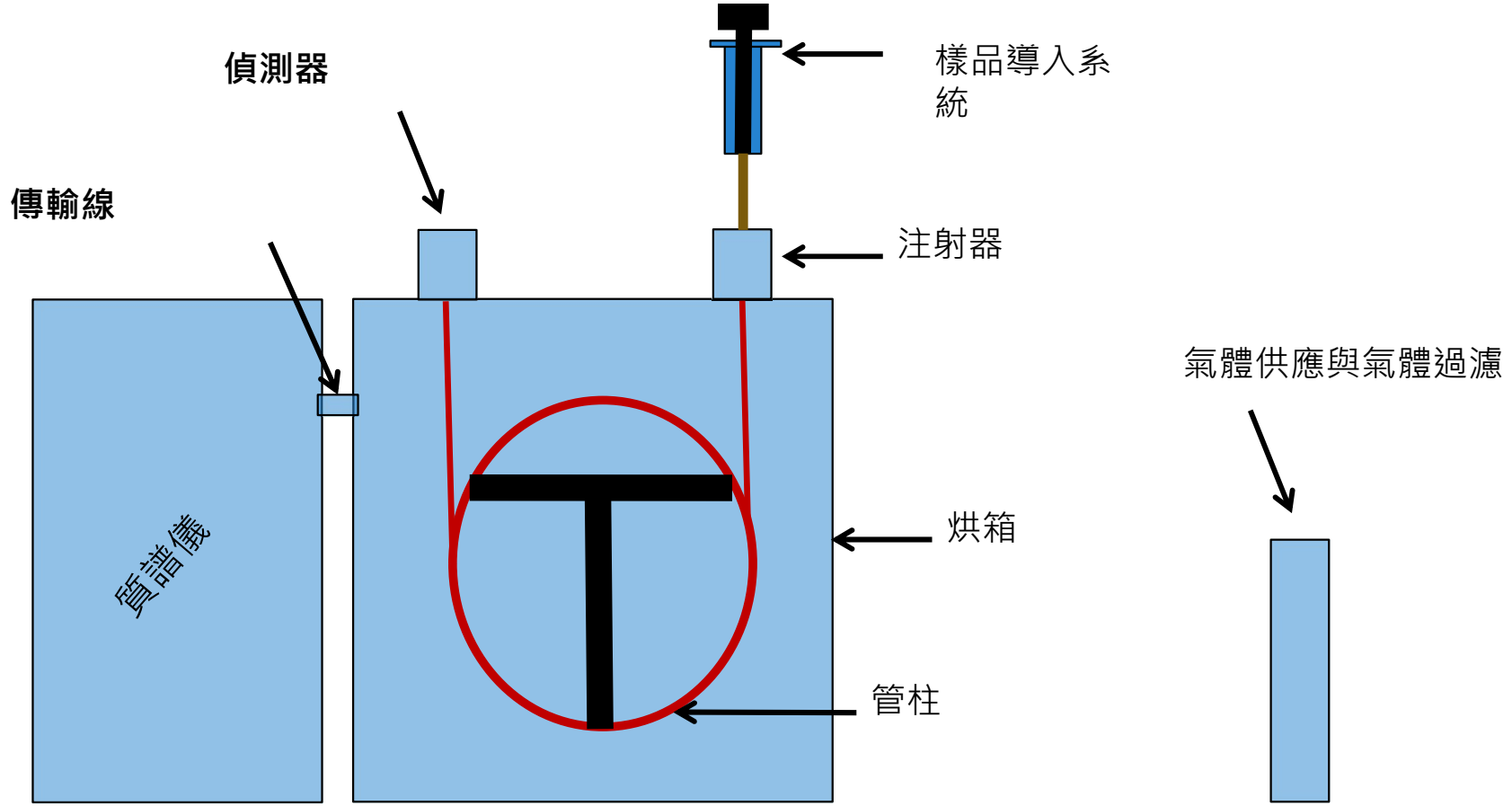
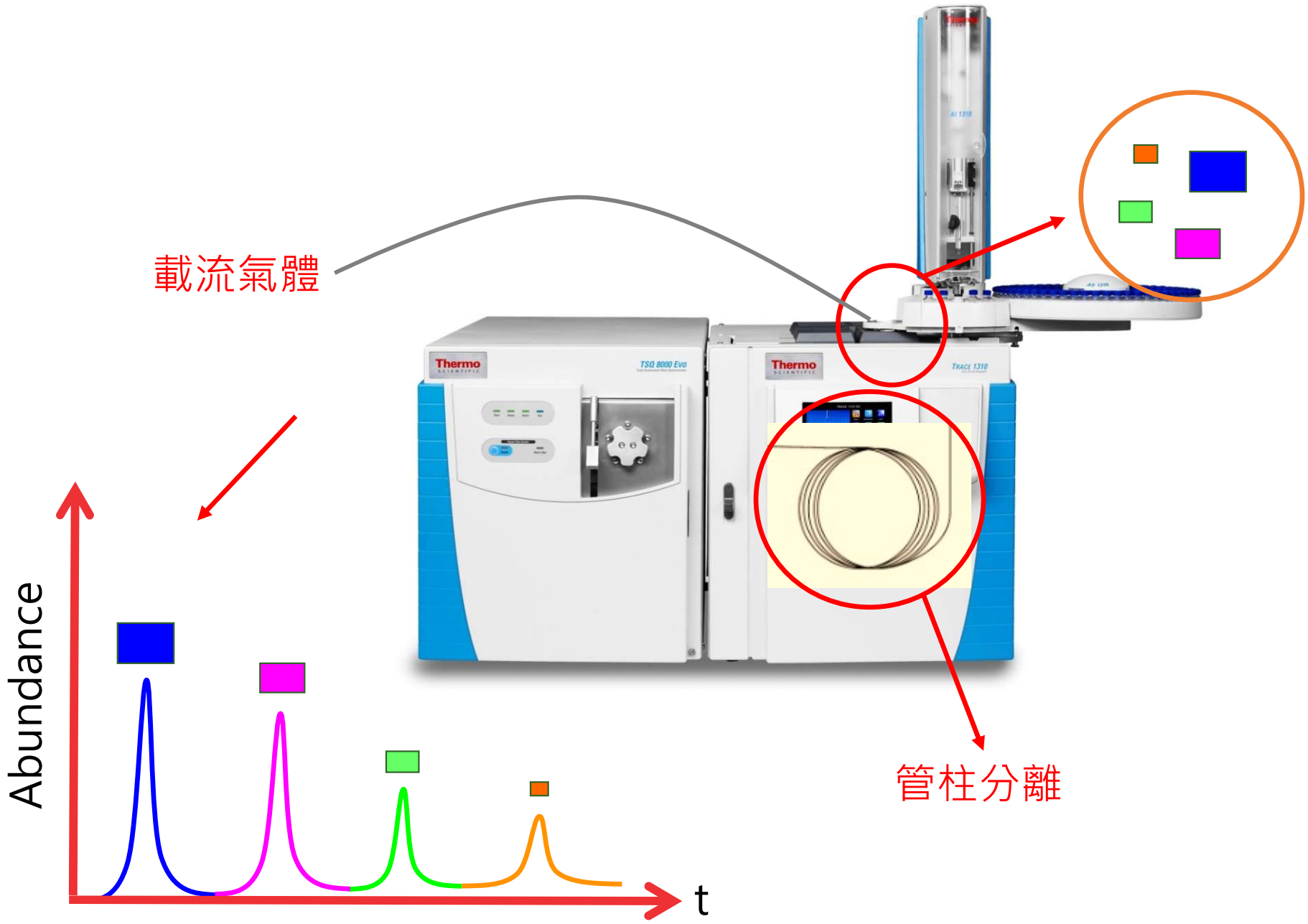
氣相層析儀組成



GC分析



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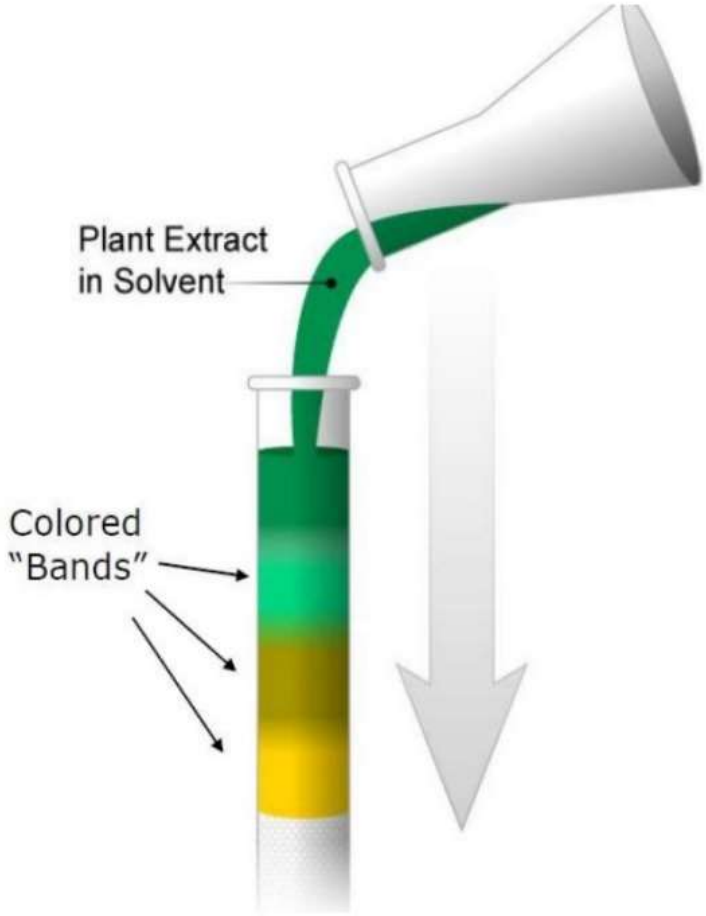
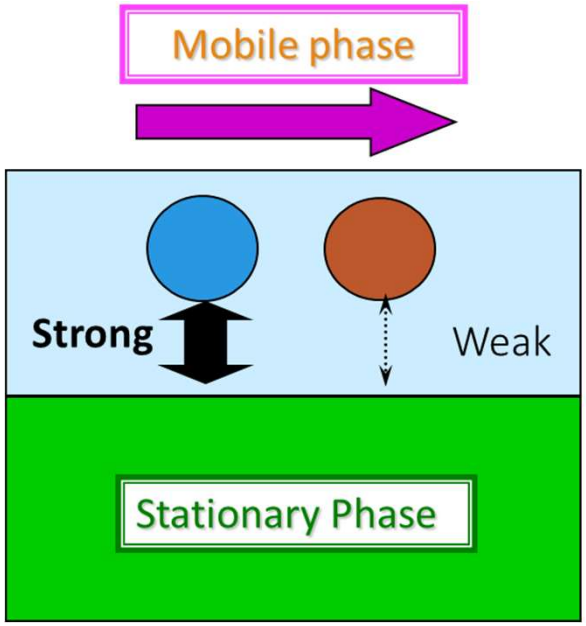
Chromatography history-層析分離原理

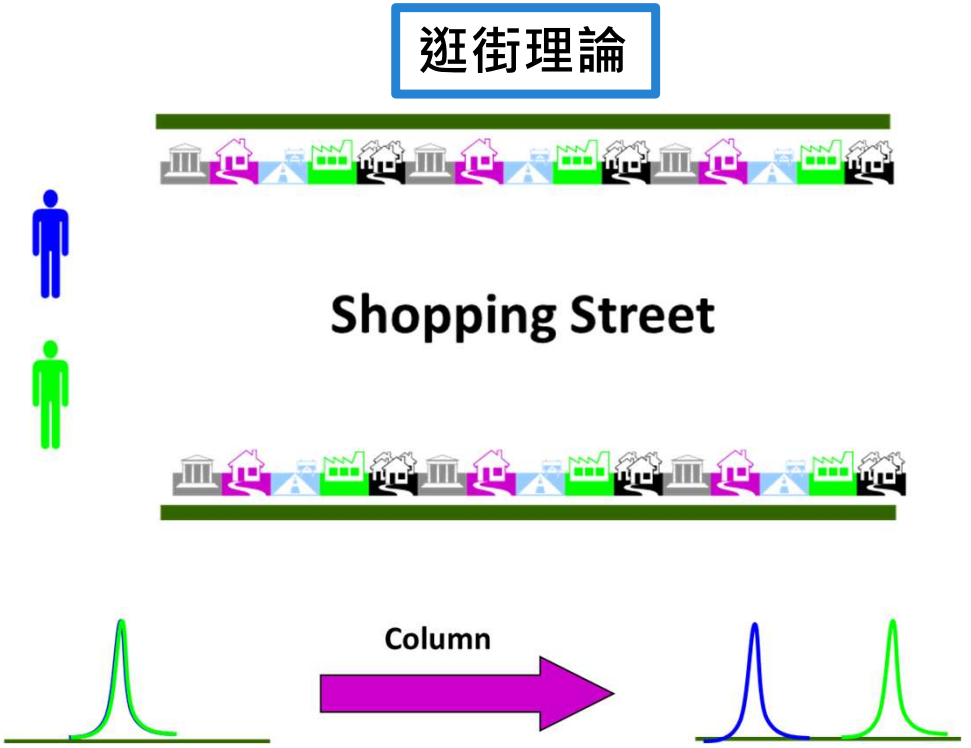
In 1903, Mikhail Twett, a Russian botanic, realized the first chromatography experiment

The word *Chromatography* is derived from greek *chroma*, which means "color", and *graphein*, which means "to write"



層析分離原理





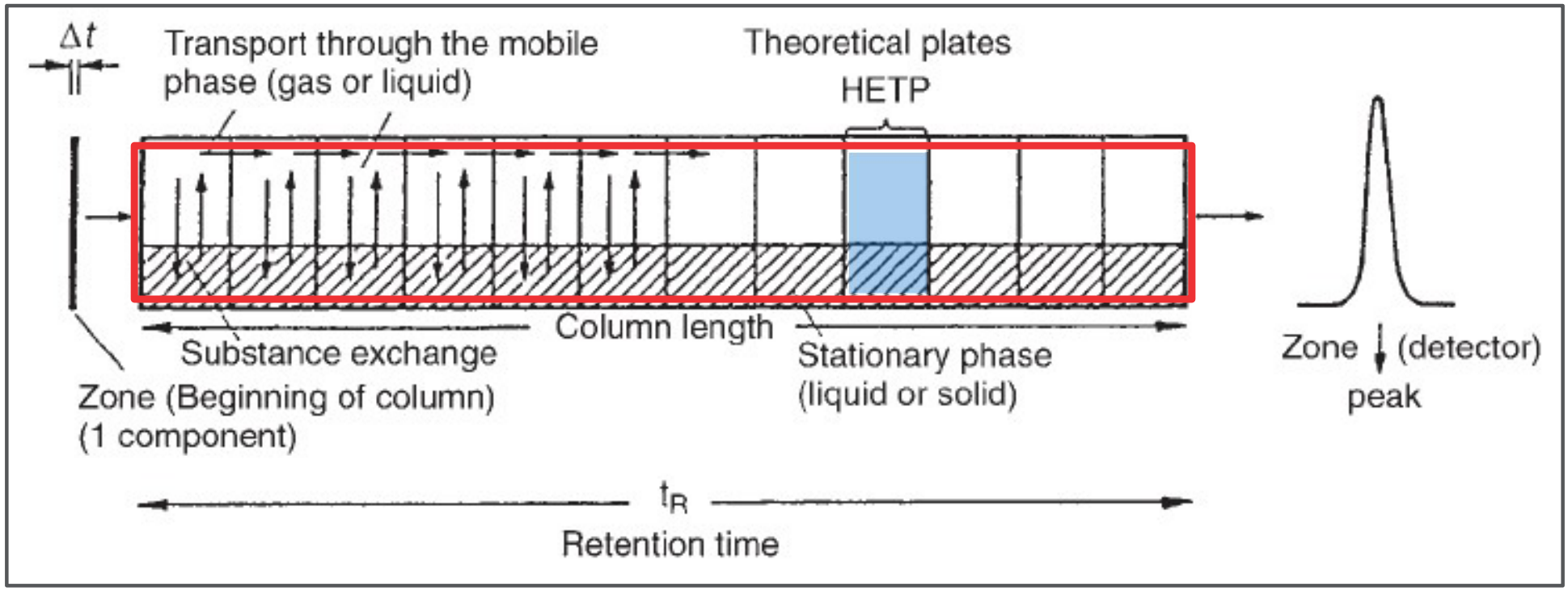
- 不同的分析物因為對於靜相與動相有不同的作用力而造成分離
- **不同的移動速度造成分離!!**

層析分離理論

- 滯留時間(RT)
- 理論板數(N)與理論板高(H)
- 滯留因子(k’)與分離因子(α)

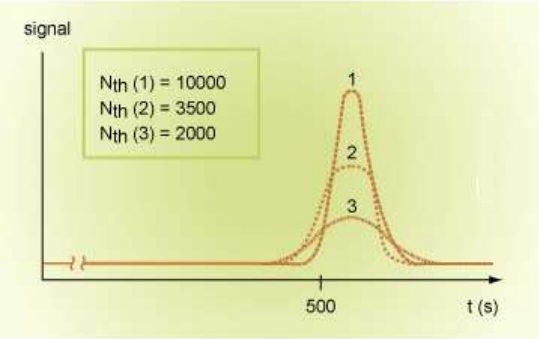
理論板高 (HETP)

- HETP: Height equivalent of theoretical plates or plate height



$$H = \frac{L}{N}$$

L = length of column (mm)
N = number of theoretical plates



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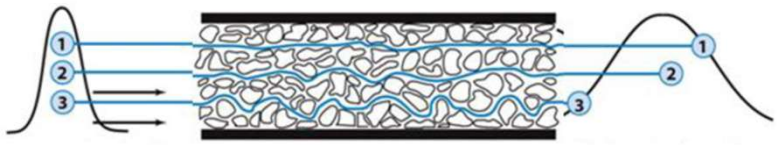
影響層析效果的三個主要因素

(A) 多重路徑(Multiple paths, Eddy diffusion)

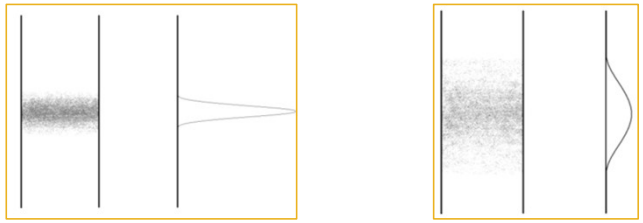
$A = 2\lambda d_p$ (d_p: particle diameter)



GC: A = 0

(B) 縱向擴散(Longitudinal diffusion)

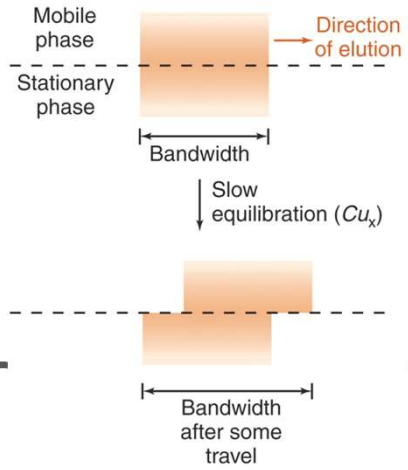
$B = 2D_m$ (D_m:diffusion coefficient)



(C) 平衡時間(Equilibration time, mass transfer)

$C = C_s + C_m$

(C_s: the rate of mass transfer through stationary phase)
(C_m: the rate of mass transfer through mobile phase)



管柱內徑、靜相膜厚

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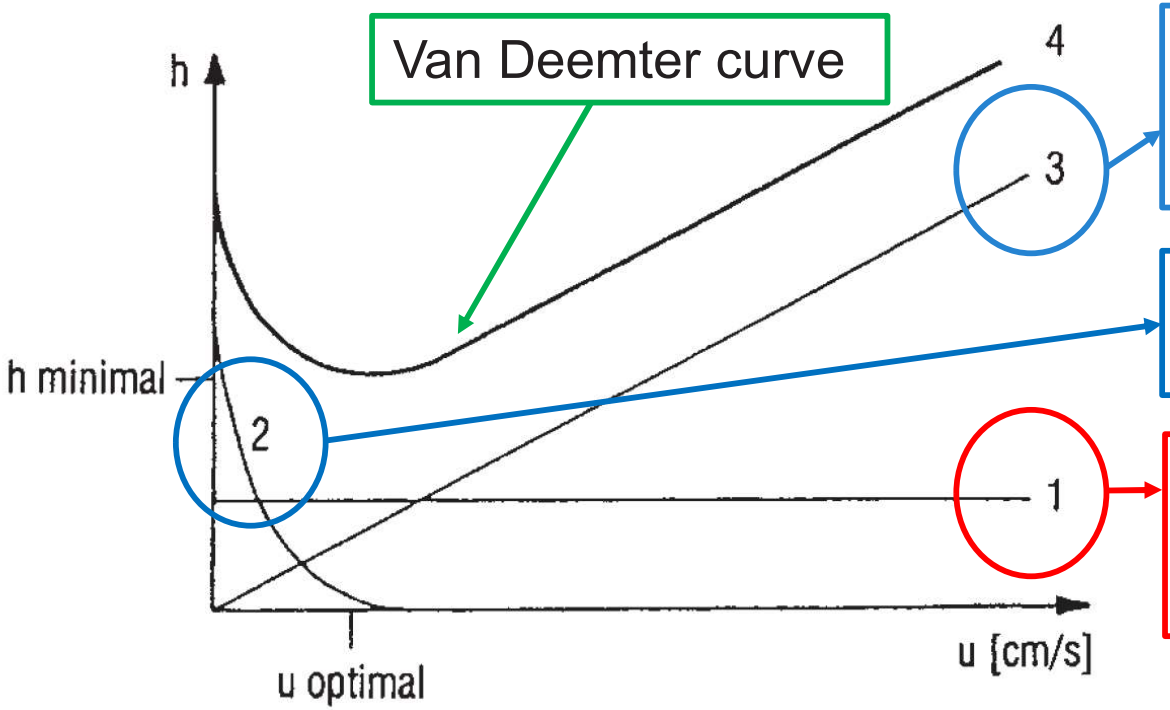
范第姆特曲線 (Van Deemter curve)



C:平衡時間
Proportion of substance exchange phenomena

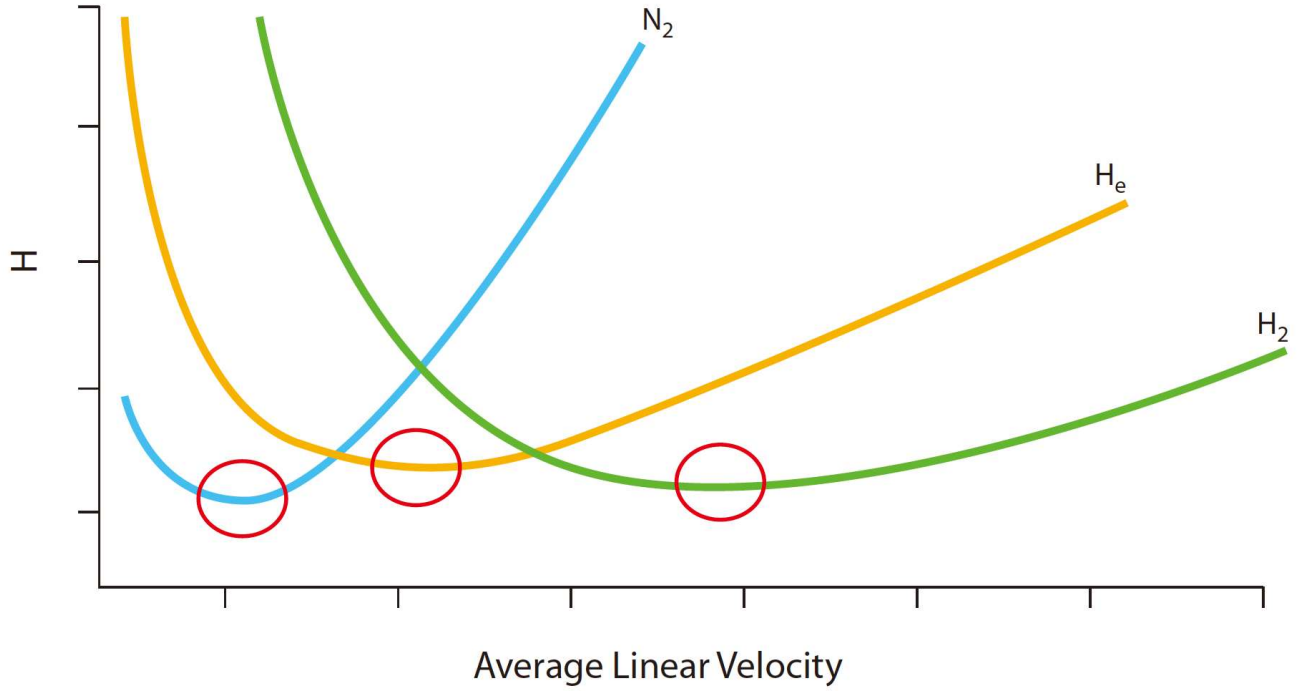
B:縱向擴散
Proportion of longitudinal diffusion

A:多重路徑
Proportion of Eddy diffusion and flow distribution at band broadening

H = A + B/u + C*u (u: 線性流速)

H = L/N

范第姆特曲線 (Van Deemter curve) / 動相組成的影響



	Column Inner Diameter (mm)					
Characteristic	**0.10**	**0.15**	**0.18**	**0.25**	**0.32**	**0.53**
Nitrogen flow (mL/min)	0.2	0.3	0.3	0.4	0.6	0.9
Helium flow (mL/min)	0.6	0.8	1.0	1.4	1.8	3.0
Hydrogen flow (mL/min)	0.7	1.1	1.3	1.8	2.3	3.7
Sample loading capacity (ng)	2.5	10	20	50	125	500
Theoretical plates/meter	11,000	7,000	6,000	4,000	3,000	2,000

Note: Flows listed are for maximum efficiency. Sample loading capacities are estimates only. Actual sample loading capacity varies with film thickness and analyte.

層析分離中解析度的定義

- 解析度: 相鄰兩個波峰的距離

$$R = \frac{1}{4}\sqrt{N} \times \left(\frac{k}{k+1}\right) \times \left(\alpha - 1\right)$$

A measure of Efficiency.
This term is affected by:
- Length
- Inner diameter
- Carrier gas type and linear velocity

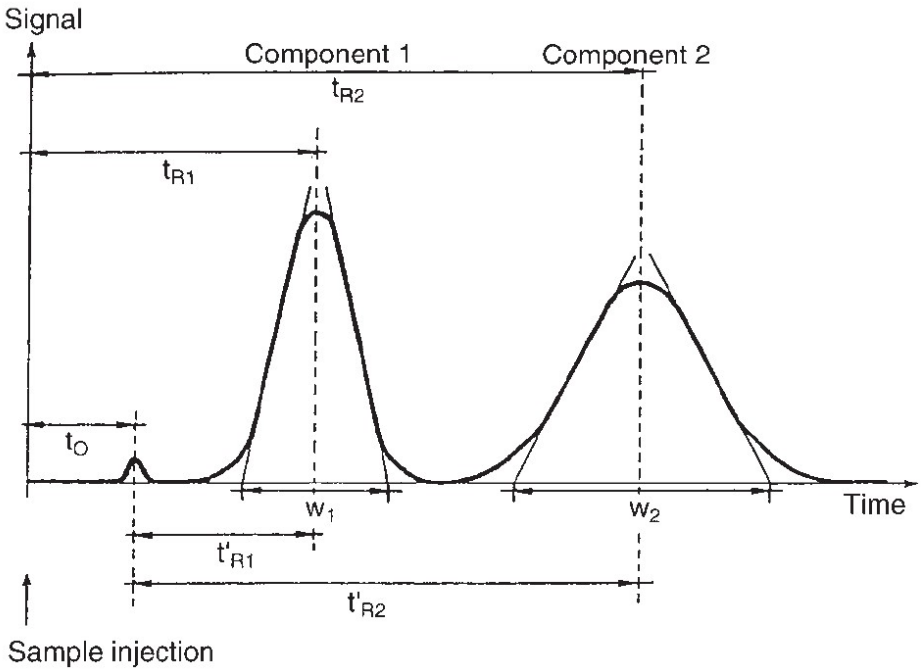
A measure of Retention.
This term is affected by:
- Inner diameter
- Film thickness
- Temperature

A measure of Peak Separation.
This term is affected by:
- Stationary phase composition
- Temperature

N = L/H = Effective theoretical plate number
L = Column length
H = HETP = Height equivalent to a theoretical plate

k = Retention factor
α = Separation factor
Baseline resolution (R = 1.5) is the goal.

滯留因子(retention factor)與分離因子(separation factor)



滯留因子: component 1

$$k' = \frac{t'_R}{t_0} = \frac{t_R - t_0}{t_0}$$

分離因子: component 1 and 2

$$\alpha = \frac{k'_2}{k'_1} = \frac{K_2}{K_1} \qquad (k'_2 > k'_1)$$

若分離因子 = 1,
表示components 1與2 並沒有分離開，因為具有相同的k’ 值

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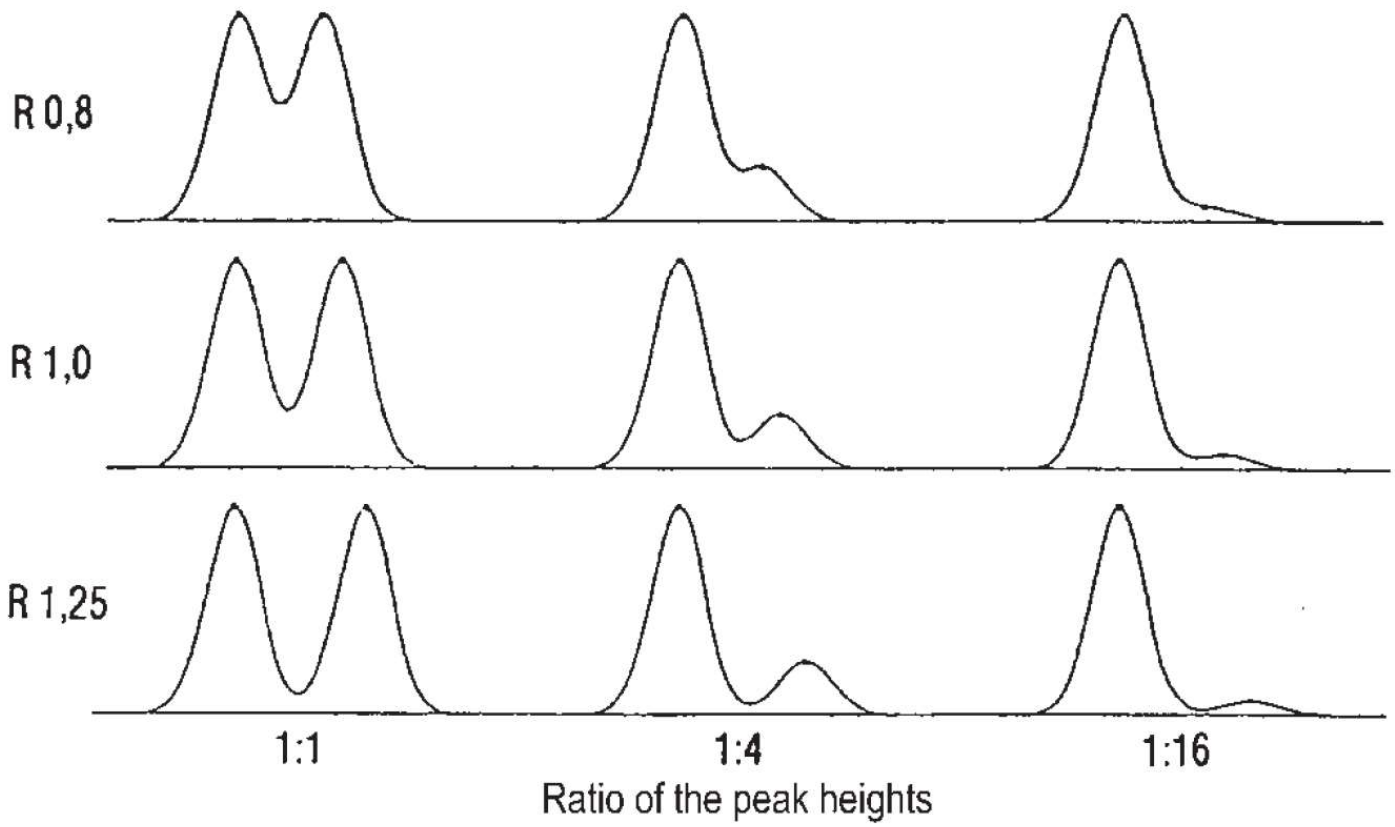
解析度

- 解析度: 相鄰兩個波峰的距離



目標: R = 1.5

GC 管柱

Like dissolves like

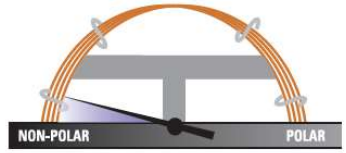
GC columns

Column Parameter	Parameters Affecting Resolution			Performance Changes
	Efficiency	Retention	Selectivity	
Column Length (m)	✔			Doubling column length increases resolution by ~ 40%
Internal Diameter (mm)	✔	✔		The smaller the column ID, the greater the efficiency and better the resolution
Film Thickness (µm)		✔		The thicker the film, the greater the retention, e.g. ideal for highly volatile compounds. The thinner the film, the sharper the peaks and lower the bleed
Stationary Phase Chemistry			✔	Altering the stationary phase can affect elution order and help separate closely, or co-eluting peaks

TraceGOLD TG-5MS GC columns

The most widely used MS phase in gas chromatography

- Low polarity phase
- Low bleed for excellent signal-to-noise ratio, sensitivity and mass spectral integrity
- Exceptional inertness ideal for analysis of active compounds
- Equivalent to USP G27 phase



Phase:	5% diphenyl / 95% dimethyl polysiloxane
Max. Temps.:	330°C/350°C for Standard Columns 430°C for Metal Columns
USP Listing:	G27, G36

TraceGOLD TG-5MS GC columns

ID (mm)	Length (m)	Film Thickness (µm)	Cat. No.	Quantity
0.10	10	0.1	26098-0200	1 Each
0.15	20	0.15	26098-2760	1 Each
	40	0.15	26098-2940	1 Each
0.18	20	0.18	26098-5780	1 Each
	20 with 5m SafeGuard	0.18	26098-5785	1 Each
0.25	15	0.25	26098-1300	1 Each
	15 with 5m SafeGuard	0.25	26098-1305	1 Each
	30	0.25	26098-1420	1 Each
		0.5	26098-2230	1 Each
		1.0	26098-2960	1 Each
	30 with 5m SafeGuard	0.1	26098-0475	1 Each
		0.25	26098-1425	1 Each
	60	0.25	26098-1540	1 Each
		0.5	26098-2350	1 Each
		1.0	26098-3080	1 Each
0.32	15	1.0	26098-2850	1 Each
	30	0.25	26098-1430	1 Each
		0.5	26098-2240	1 Each
		1.0	26098-2970	1 Each
	30 with 5m SafeGuard	0.25	26098-1435	1 Each
	60	0.25	26098-1550	1 Each
		0.5	26098-2360	1 Each
		1.0	26098-3090	1 Each

Applications:

- Semi-volatiles
- Phenols
- Amines
- Residual solvents and solvent impurities
- Drugs of abuse
- Pesticides
- PCB cogeners
- Aroclor mixes

Similar to:

- Rxi-5ms
- DB-5
- HP-5
- HP-5ms
- Ultra-2
- SPB-5
- Equity-5
- CP-Sil 8

Find out more at **thermofisher.com/chromatographyconsumables**

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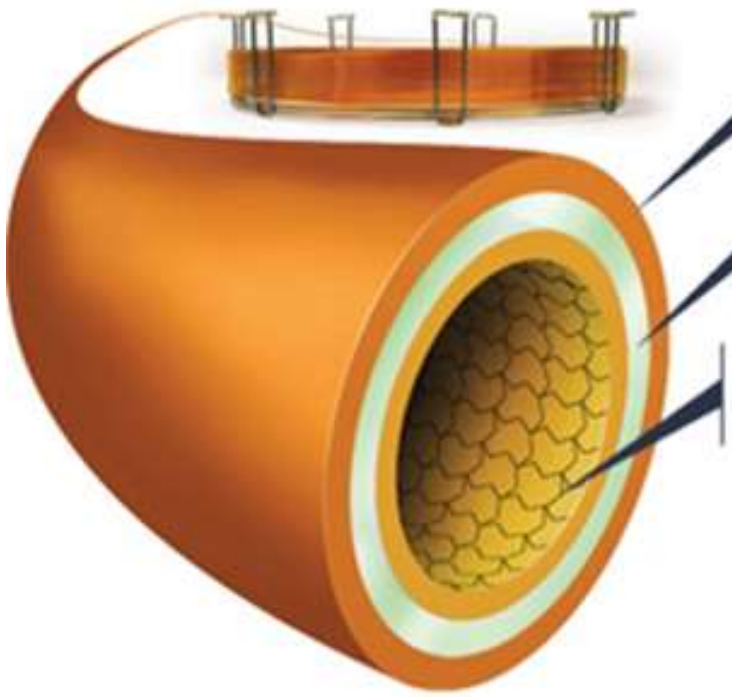
GC Capillary Column Structure

Choice of Column Dimensions



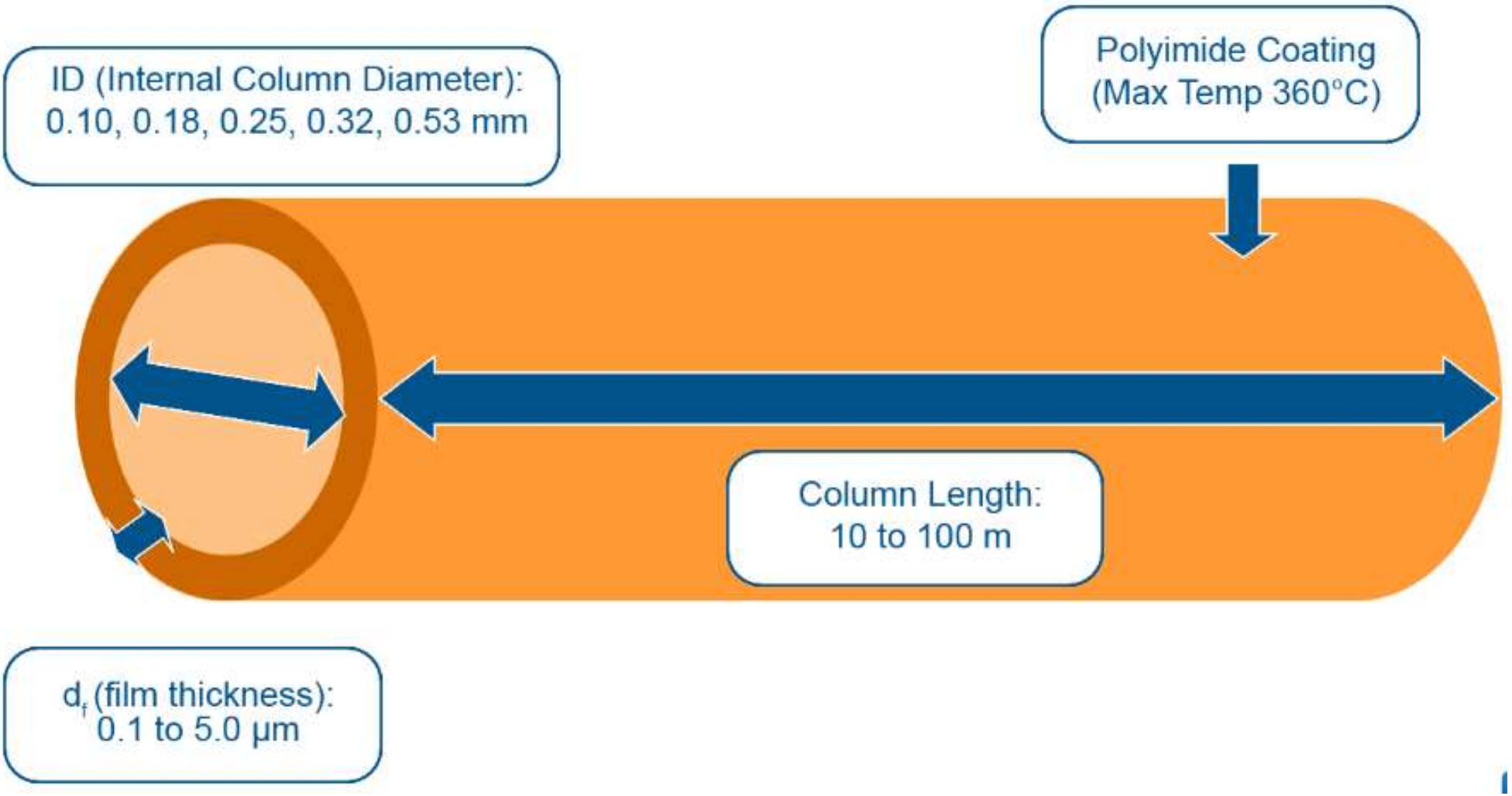
Polyimide coating
Provides strength to the fused silica and temperature stability.

Fused silica tubing
Provides the column structure for phase bonding; high temperature resistance, low reactivity.

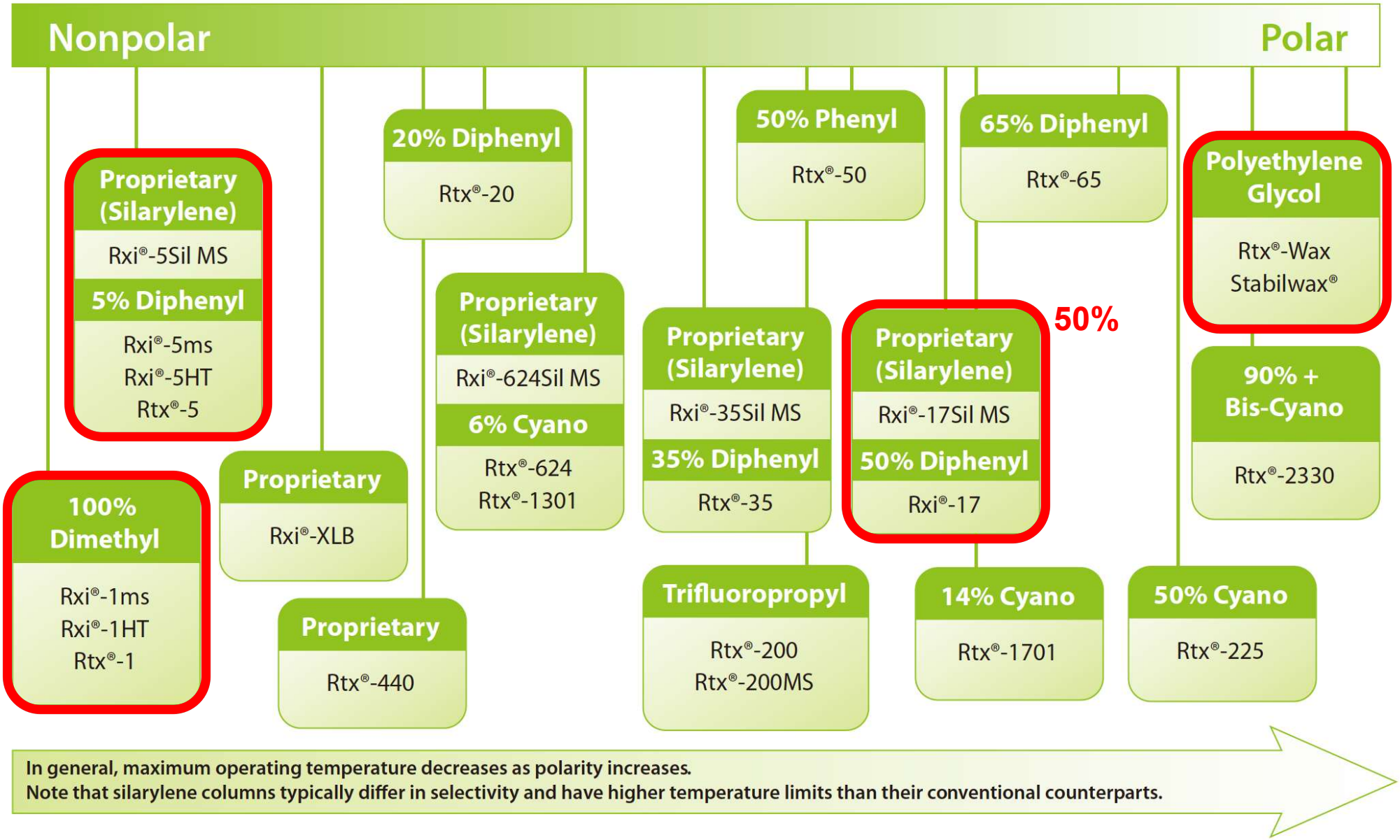
Column phase
The stationary phase. Provides the separation. Commonly comprised of Polysiloxane compounds.

For example:

5% Phenyl Phase
TG-5MS 30 m x 0.25 mm x 0.25 µm



GC管柱 / 靜相選擇



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分析管柱的選擇 / 毛細管柱

- **靜相種類:**

  - 極性分類, TG-1MS, TG-5MS, TG-17

- **內徑 (I.D.):**

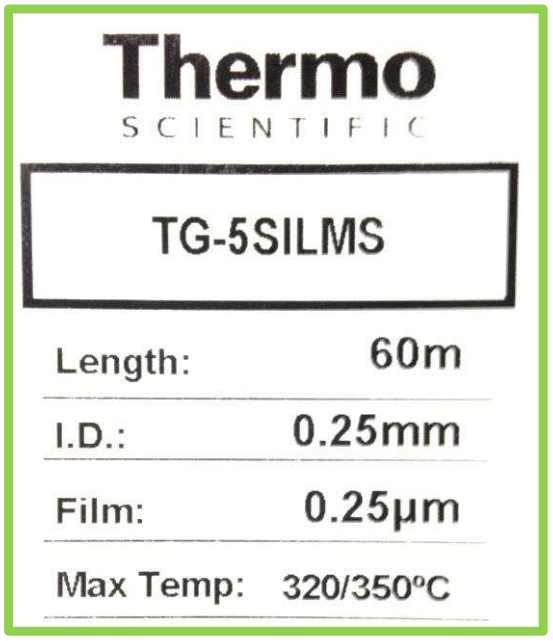
  - 0.53 mm -> GC-FID -> 3 mL/min
  - 0.32 mm -> GC-MS -> 1~3 mL/min
  - 0.25 mm -> GC-MS -> 0.5~1.5 mL/min

- **膜厚:**

  - 樣品承受量(capacity) ∝ 膜厚 ∝ 剝落 (bleeding)

- **長度:**

  - $N \propto \sqrt{L}$



Internal Diameter (mm)	Film Thickness (µm)					
	0.1	0.25	0.5	1	1.8	3
0.1	250	100	50	25	14	8
0.25	625	250	125	63	35	21
0.32	800	320	160	80	44	27
0.53	1325	530	265	133	74	44

Phase ratio:

Volatile, < 100
General, ≈ 250
High M.W., > 400

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靜相作用力

1. 分散(Dispersion)
2. 偶極(Dipole)
3. 氫鍵(Hydrogen bonding)

Functional Group	Dispersion	Dipole	Hydrogen Bonding
Methyl	Strong	None	None
Phenyl	Strong	None to Weak	Weak
Cyanopropyl	Strong	Very Strong	Moderate
Trifluoropropyl	Strong	Moderate	Weak
PEG	Strong	Strong	Moderate

氫鍵的相對強度

Strength	Compounds
Strong	Alcohols, carboxylic acids, amines
Moderate	Aldehydes, esters, ketones
Weak to None	Hydrocarbons, halocarbons, ethers

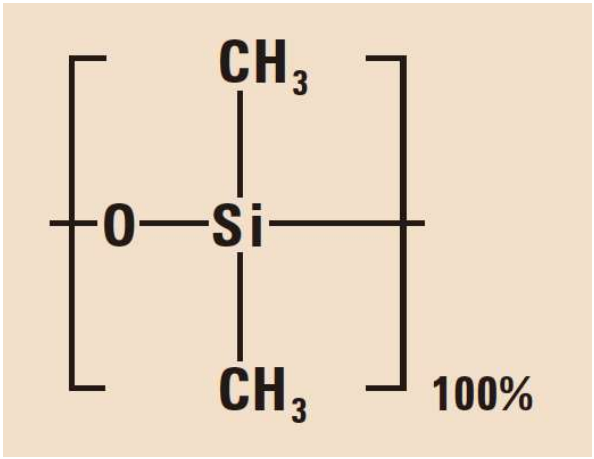
GC管柱 / 靜相作用力

分散(Dispersion) – 主要的作用力

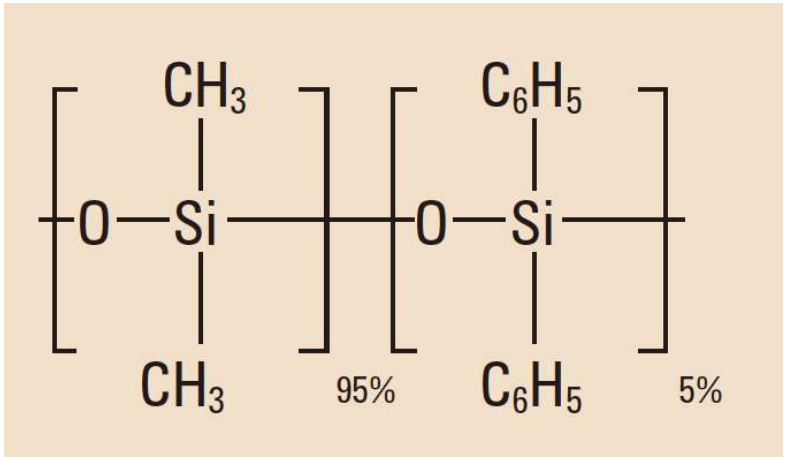
1. 所有的靜相皆有此作用力，如: 聚矽氧烷 (polysiloxane) 與 聚乙二醇 (polyethylene glycol)
2. 可以簡單的使用揮發性(volatility)與沸點 (boiling point)來判斷作用力大小
3. 須注意的是不可以單單使用沸點來做判斷
4. 沸點基本上只可應用在分析物具有**相似結構/官能基或是同系物**
5. 若沸點差異 ≥ 30 °C，通常就可以使用絕大部分的靜相分離開
   若沸點差異 ≤ 10°C，用沸點的判斷方式就很可能會出錯

GC管柱 / 靜相結構

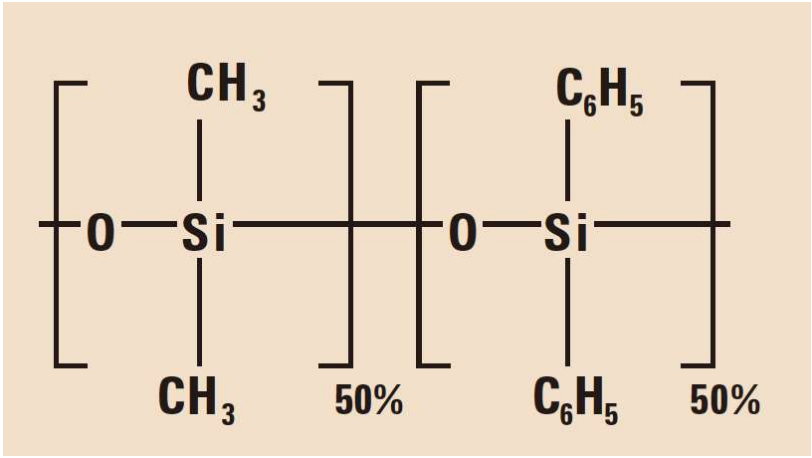
DB-1



DB-5



DB-17



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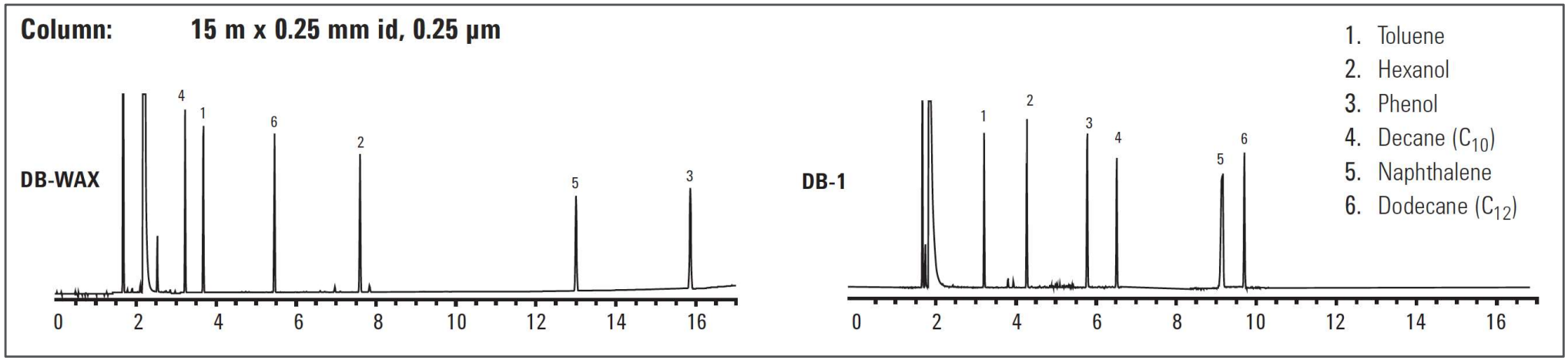
GC管柱 / 靜相作用力

氫鍵:

發生在分析物跟靜相具有可以產生氫鍵的官能基上(-OH, -NH_2)



高極性

- WAX

4 > 1 > 6 > 2 > 5 > 3

低極性

- 100% dimethyl

1 > 2 > 3 > 4 > 5 > 6

GC管柱 / 內徑(I.D.)

Small ID → **Large ID**

0.15 mm–0.18 mm

Characteristics
- Highest efficiency
- Shorter analysis time
- Lower sample loading capacity

Applications
- Highly complex samples
- Fast GC
- GC-MS
- Split injection

0.25 mm–0.32 mm

Characteristics
- High efficiency
- Good performance for analysis time and sample loading capacity

Applications
- Complex samples
- Wide concentration range
- Split , splitless, direct, headspace, and on-column injection

0.53 mm

Characteristics
- Good efficiency
- Longer analysis time
- Higher sample loading capacity
- May require higher flow rates than MS detectors can tolerate

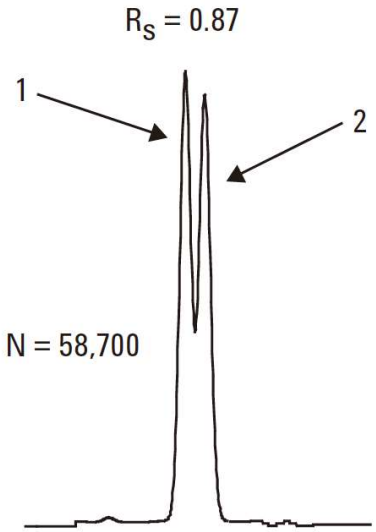
Applications
- Packed column replacement
- Purity analysis
- Split, splitless, direct, headspace, and on-column injection

As inner diameter increases, efficiency decreases, sample loading capacity increases, optimal flow rate increases, and analysis time increases.

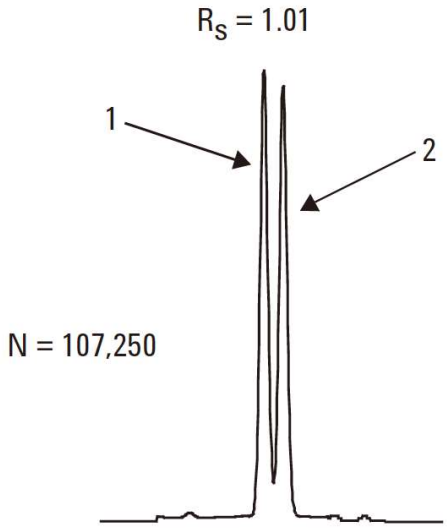
GC管柱 / 內徑(I.D.)

Column: **DB-624, 30 m**

1. 1,3-Dichlorobenzene
2. 1,4-Dichlorobenzene



0.53 mm I.D., 3.0 µm



0.32 mm I.D., 1.8 µm

Column ID Diameter (mm)	Theoretical Plates/Meter
0.10	12,500
0.18	6,600
0.20	5,940
0.25	4,750
0.32	3,710
0.45	2,640
0.53	2,240

Maximum efficiency for a solute with k=5

GC管柱 / 內徑(I.D.)

Inside Diameter	Resolution	Speed	Capacity	Ease
 100 μm	Very good	Very good	Fair	Fair
 250 μm 320 μm	Good	Good	Good	Good
 530 μm	Fair	Good	Very good	Very good

GC管柱 / 靜相膜厚

Thin Film — **Thick Film**

0.10 µm–0.50 µm

Characteristics
- Shorter retention times
- Lower bleed
- Higher maximum temperatures
- Lower sample loading capacity
- High resolution for high molecular weight compounds

Applications
Medium and high molecular weight compounds

1.0 µm–10.0 µm

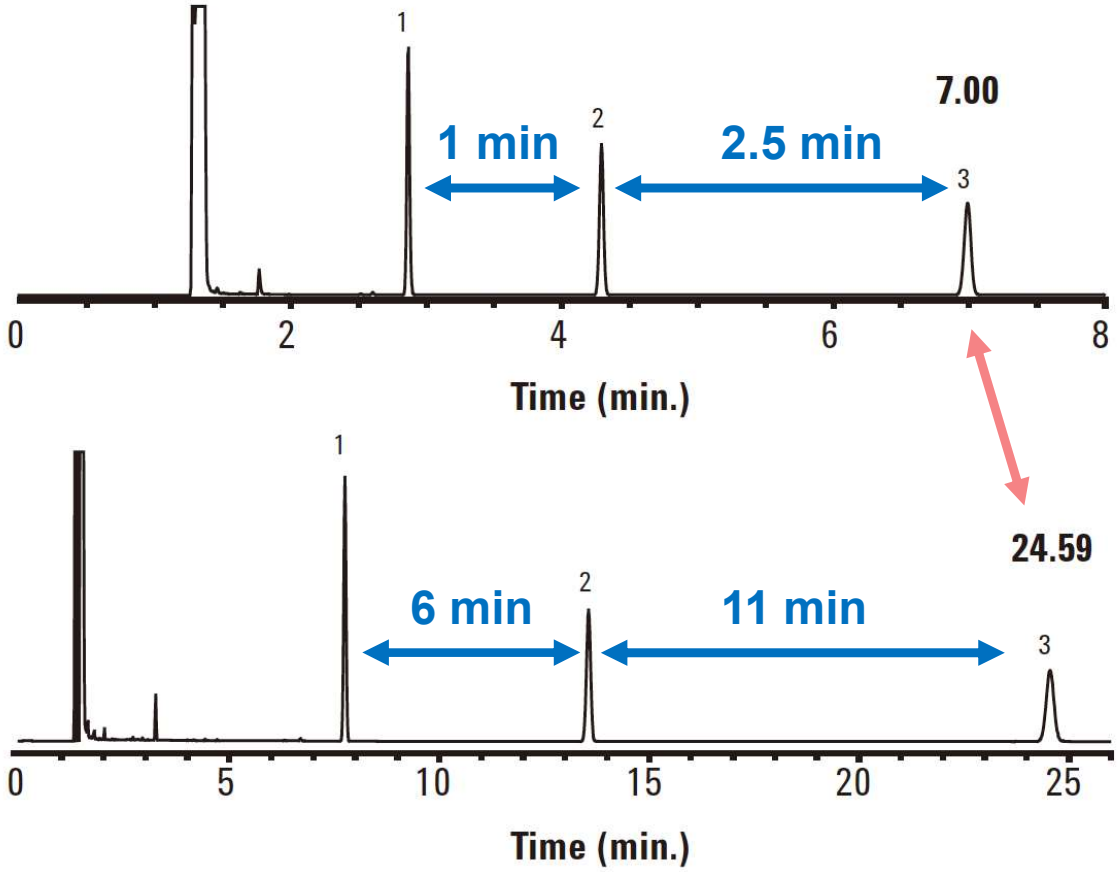
Characteristics
- Longer retention times
- Higher bleed
- Lower maximum temperatures
- Higher sample loading capacity
- High resolution for volatiles and low molecular weight compounds

Applications
- Volatile, low molecular weight compounds
- High concentration samples (e.g., purity testing)

As film thickness increases, retention, sample loading capacity, and column bleed increase; whereas, maximum temperature decreases.

GC管柱 / 靜相膜厚

Column: DB-1, 30 m x 0.32 mm I.D.
Carrier: Helium at 38 cm/sec
Oven: 100 °C isothermal



1. n-Decane
2. n-Undecane
3. n-Dodecane

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GC管柱 / 管柱長度

Short Length → **Long Length**

5 m–15 m

Characteristics
- Good efficiency
- Short analysis times

Applications
Samples with few compounds

20 m–30 m

Characteristics
- Better efficiency
- Moderate analysis times

Applications
More complex samples

50 m–150 m

Characteristics
- Best efficiency
- Longer analysis times

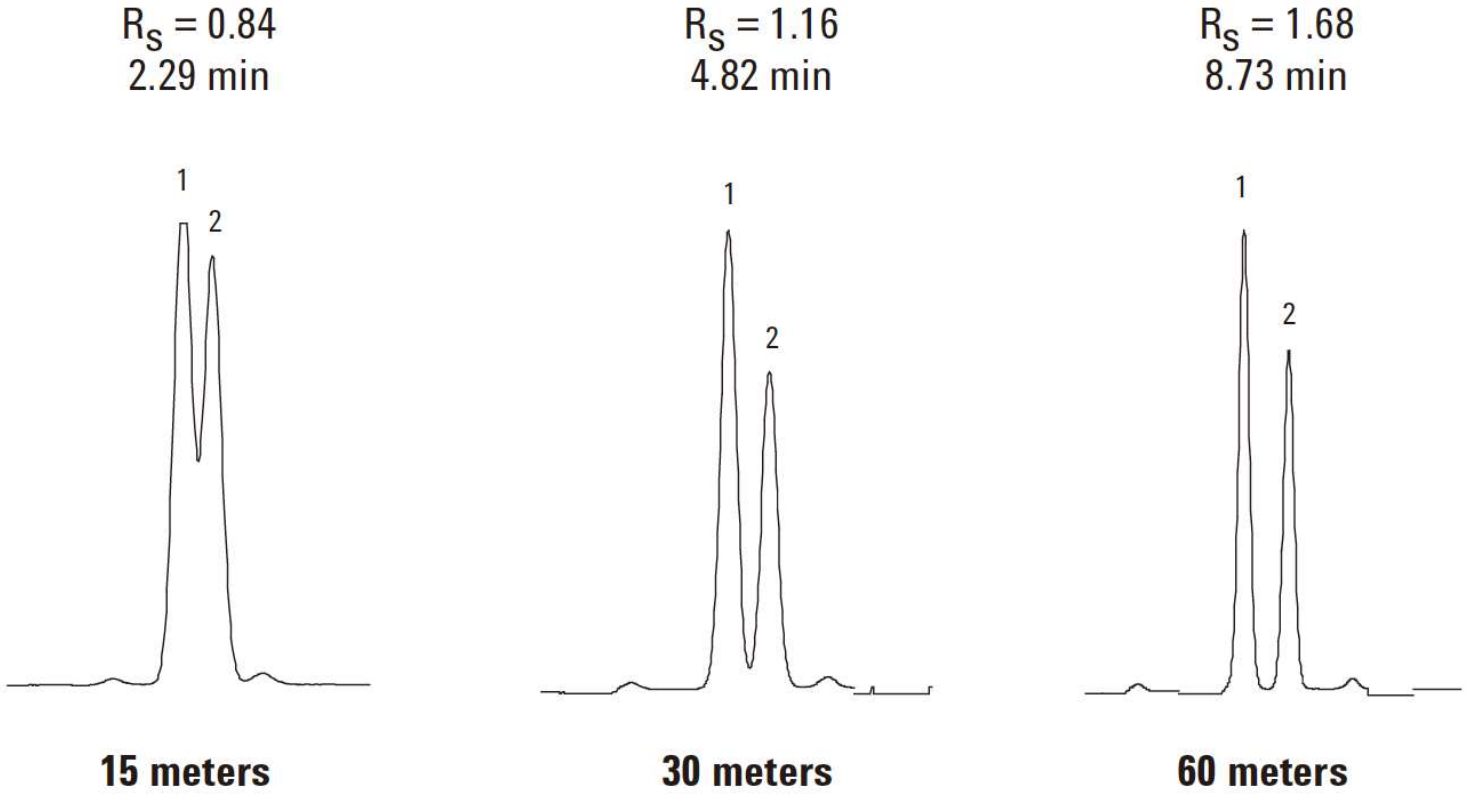
Applications
Very complex samples

Longer Columns Can Increase Resolution...
Doubling the column length only increases resolution by approximately 40% because the column length is under the square root function in the efficiency term of the resolution equation.

But, Longer Columns Increase Cost and Analysis Time
On longer columns, analysis time is increased by as much as a factor of two.
Longer columns are also more expensive.

GC管柱 / 管柱長度

- $N \propto \sqrt{L}$



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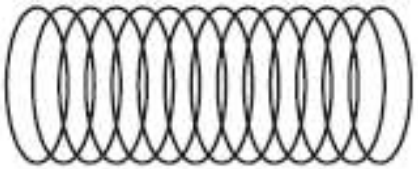
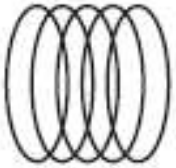
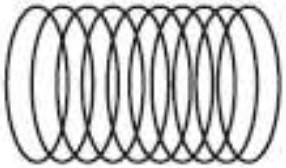
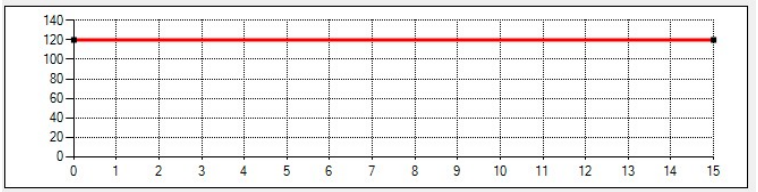
GC管柱 / 管柱長度

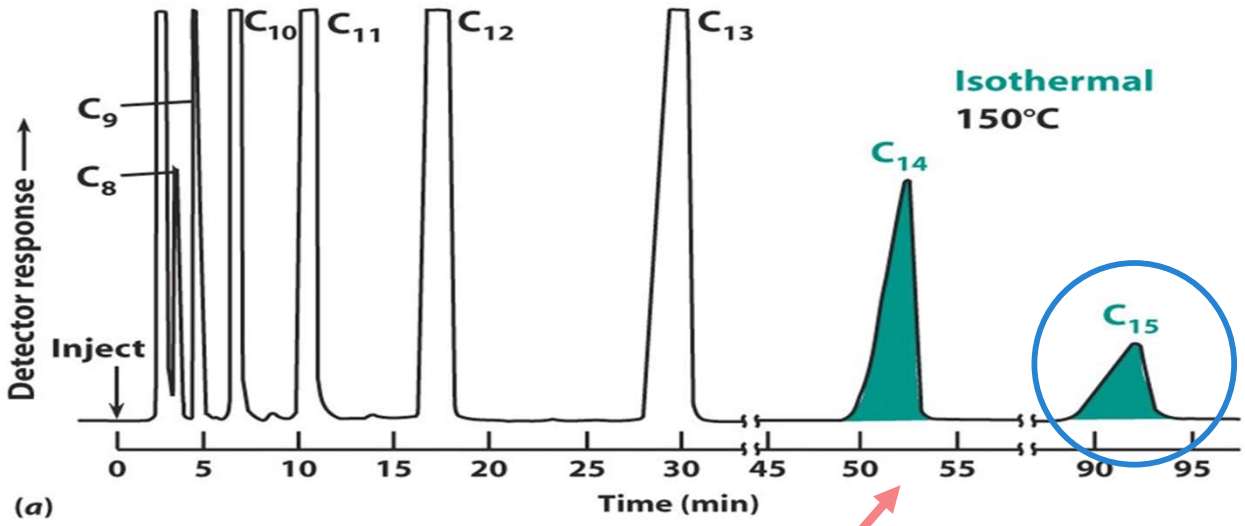
- $N \propto \sqrt{L}$

Column Length	Resolution	Speed
 Long (60–100 m)	High	Slow
 Short (5–10 m)	Moderate	Fast
 Medium (25–30 m)	Good compromise, good starting point	

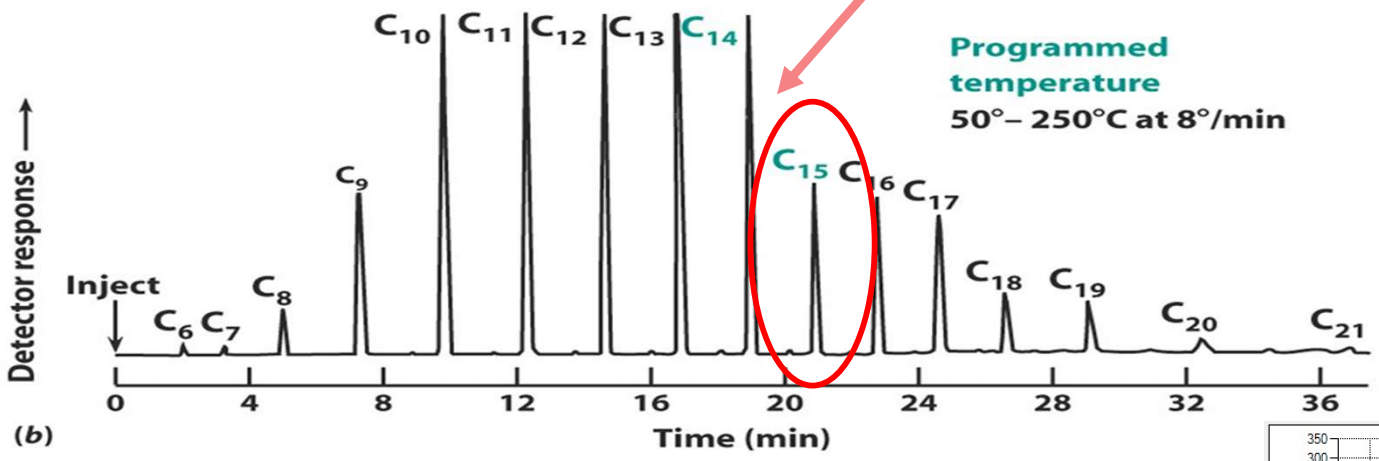
GC的層析分離方式 / 恆溫與程序升溫

1. 恆溫 (Isothermal)

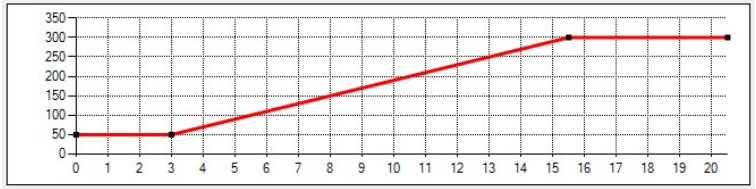




- 節省時間
- 改善波峰形狀



2. 程序升溫 (Temperature programming)



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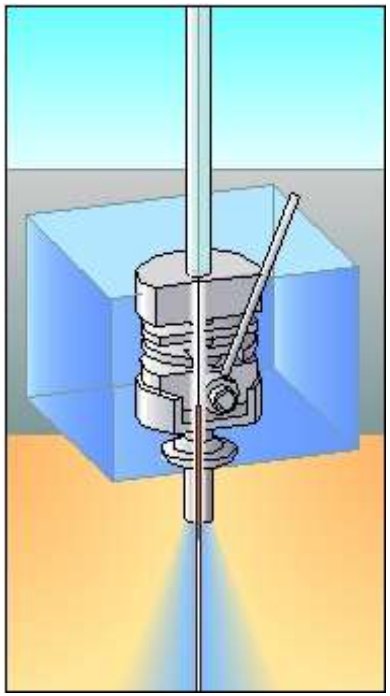
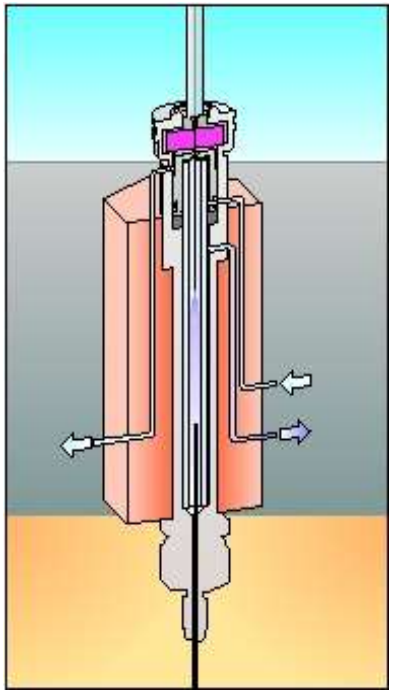
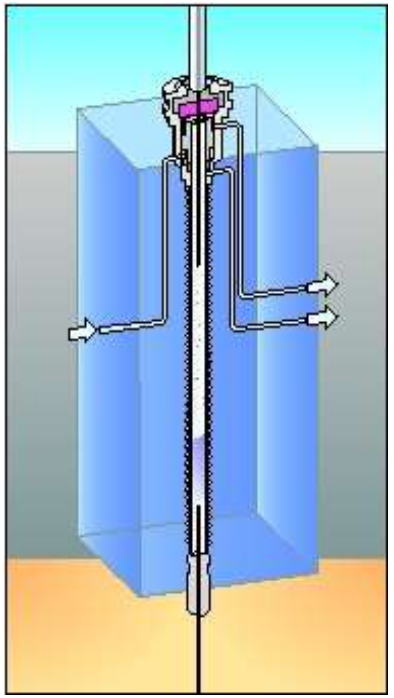
GC進樣口



On-column

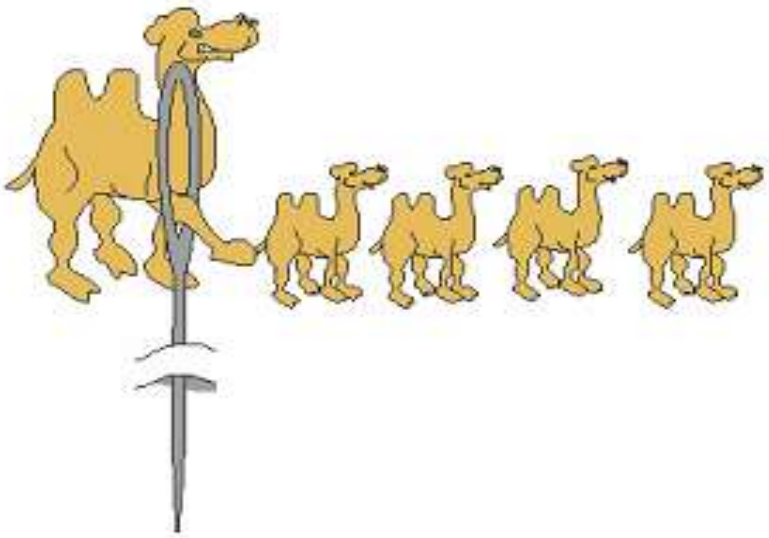


Split/Splitless

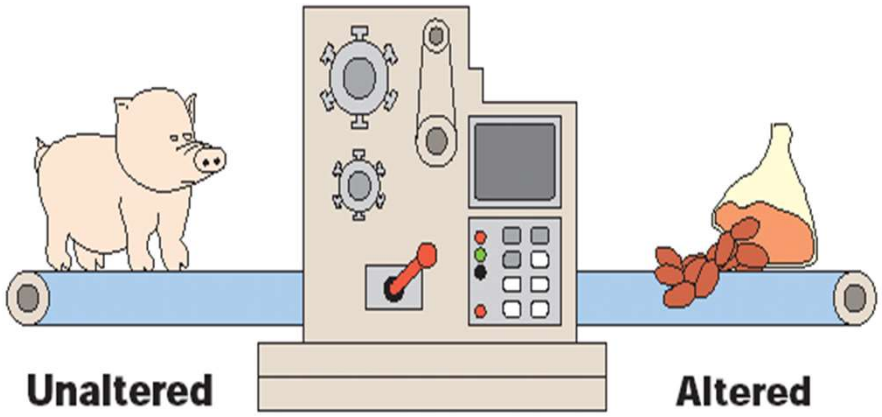


PTV

GC進樣口注意事項

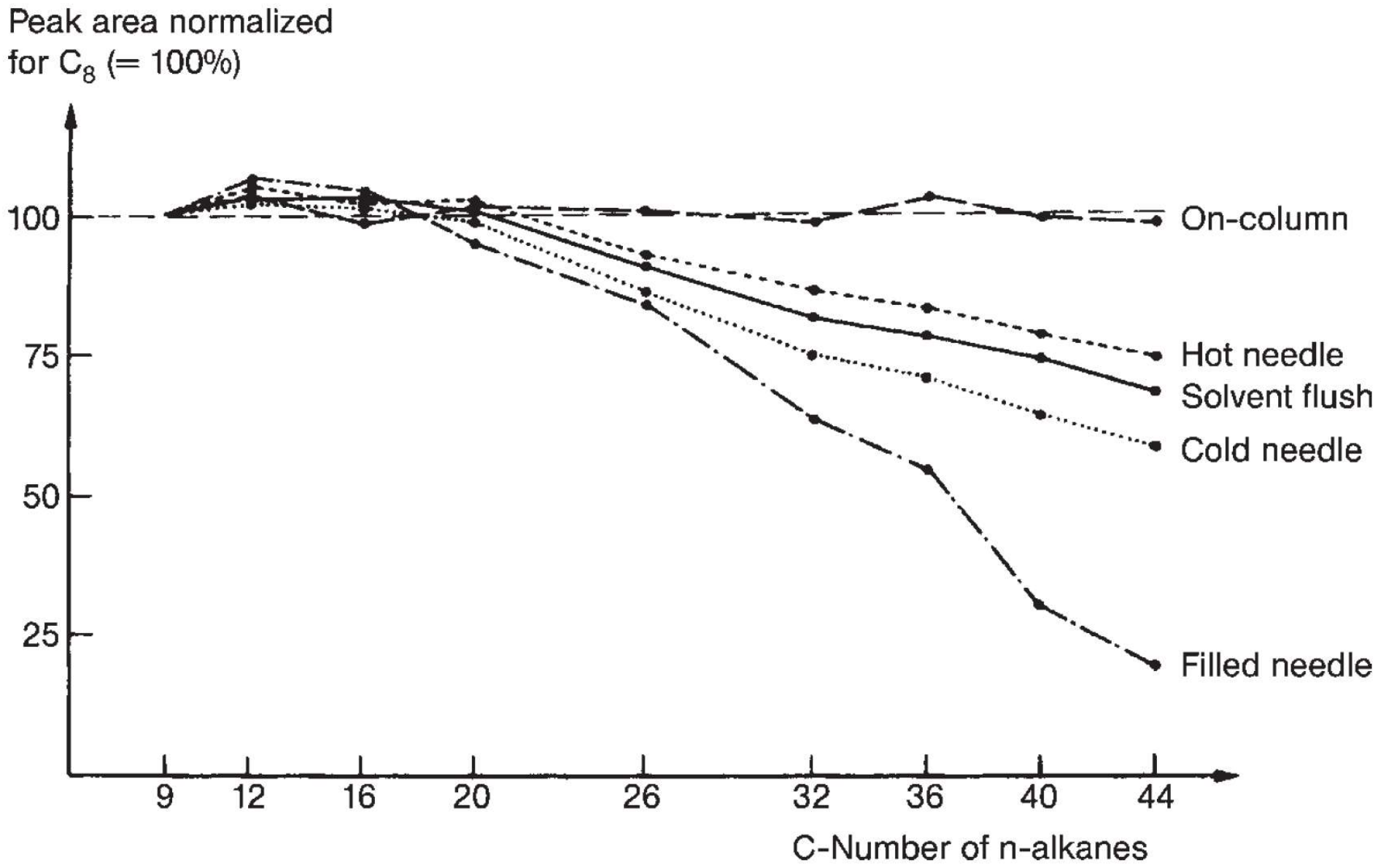


- 質量歧視效應



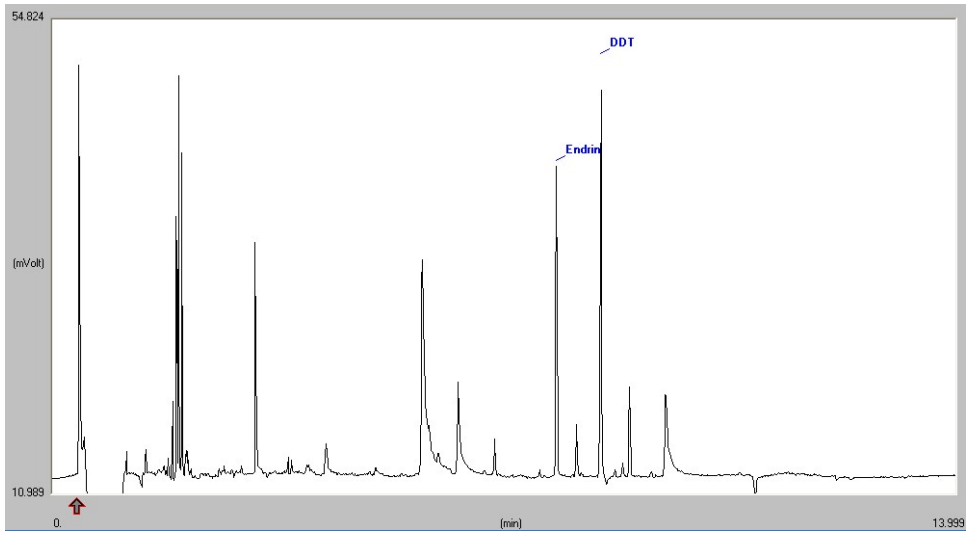
- 熱不穩定

質量歧視效應



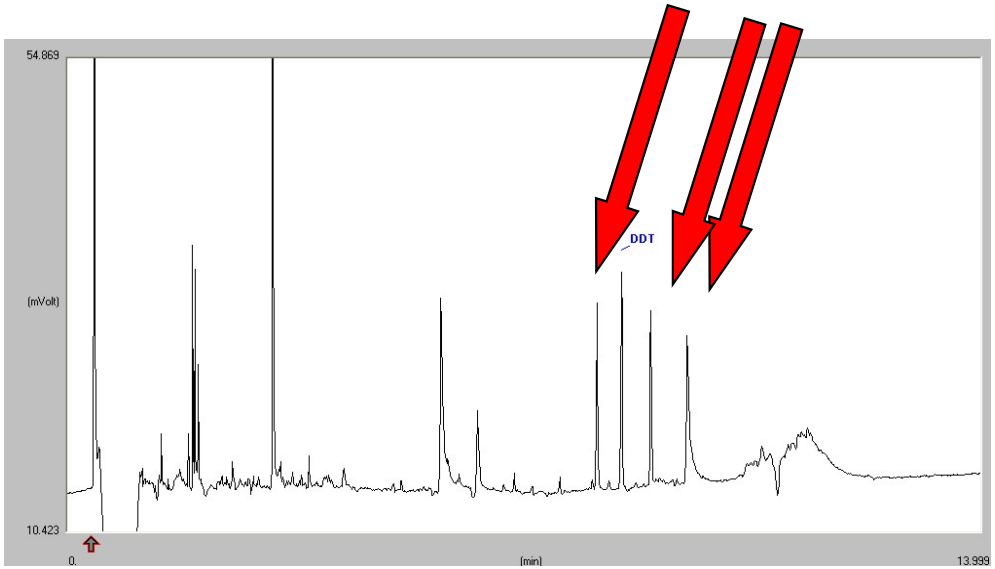
熱不穩定

- 2 ul solution 1 ppb Endrin/DDT solution with ECD detector



Good deactivation

Degradation byproducts



Bad deactivation

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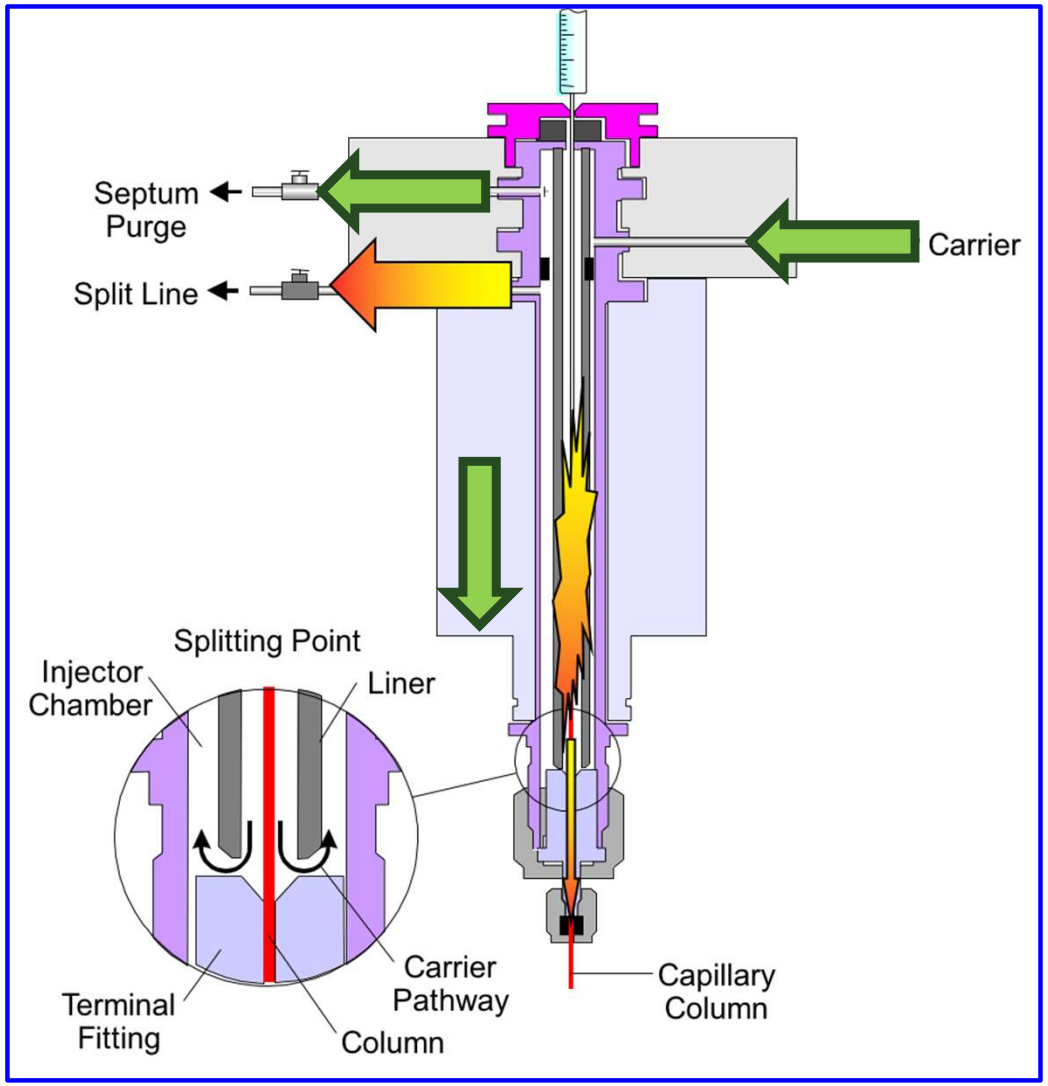
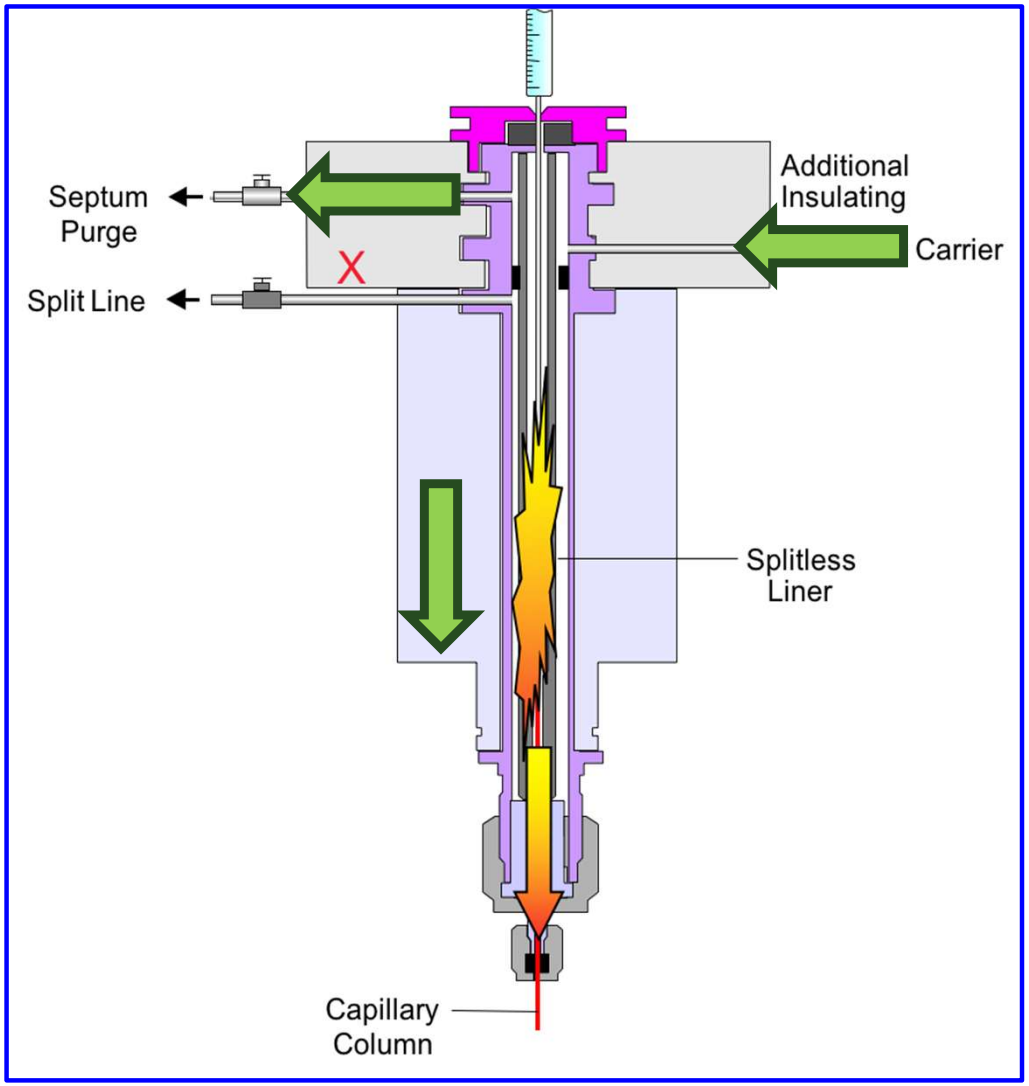
進樣模式1: 分流/不分流注射口 (SSL injector)



分流 (Split)



不分流 (Splitless)
微量分析用

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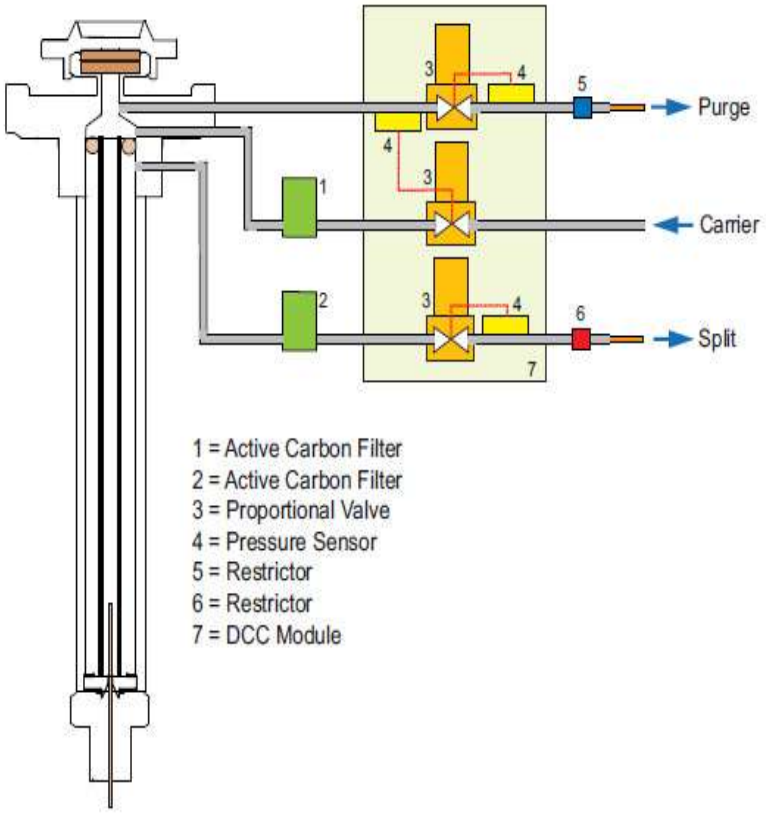
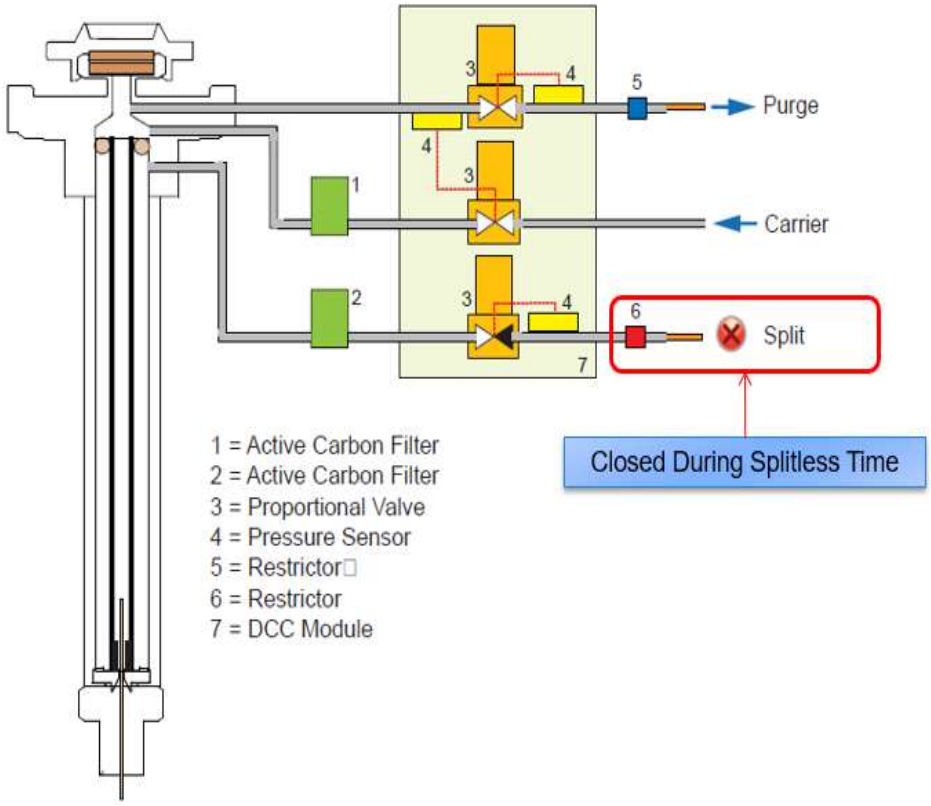
注射器- 分流/不分流(SSL)





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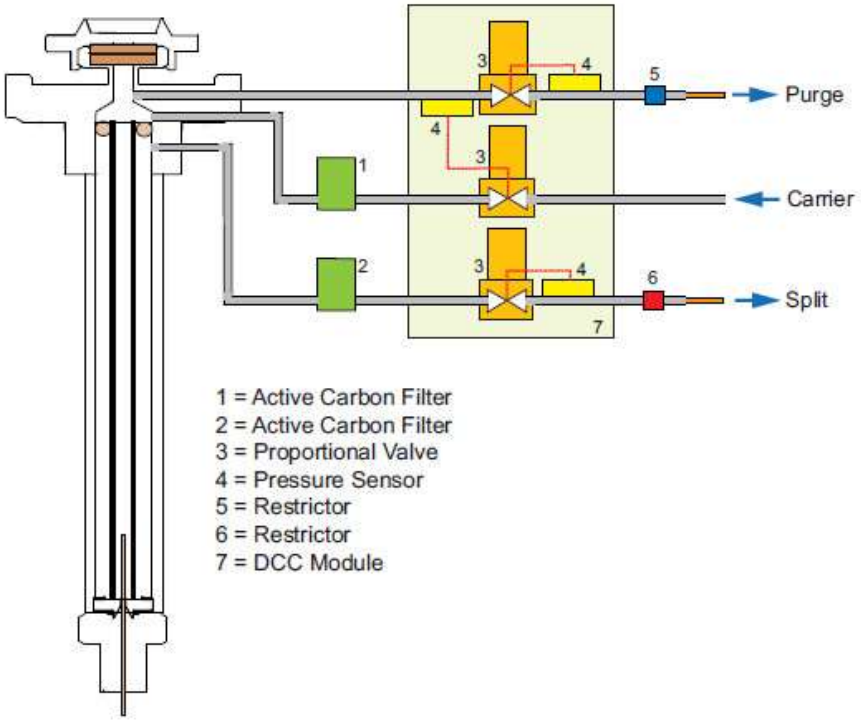
注射器- 分流/不分流(SSL)



分流注射 Split injection

當樣品濃度過高或是無須關注過低濃度不純物樣品(不考慮LOD)。分流進樣可以通過一些流量(樣品與載氣)進入GC的管柱其餘的流量則會通過分流控制閥而不進入管柱

不分流注射 Splitless injection

當樣品中的待測目標化合物濃度含量比較低時。分流控制閥會先關閉，讓待測的樣品於一定時間進入管柱中，然後再開打分流控制閥利用大量的氣體來沖洗掉人和殘留在汽化管中的溶劑或是樣品

分流比 Split Ratio

- 分流比計算：載流氣體進入管柱與通過分流控制閥比例

- 分流的設定對於層析波峰的形狀與分析的靈敏度有著關鍵的影響

Split vent flow / column flow = split ratio

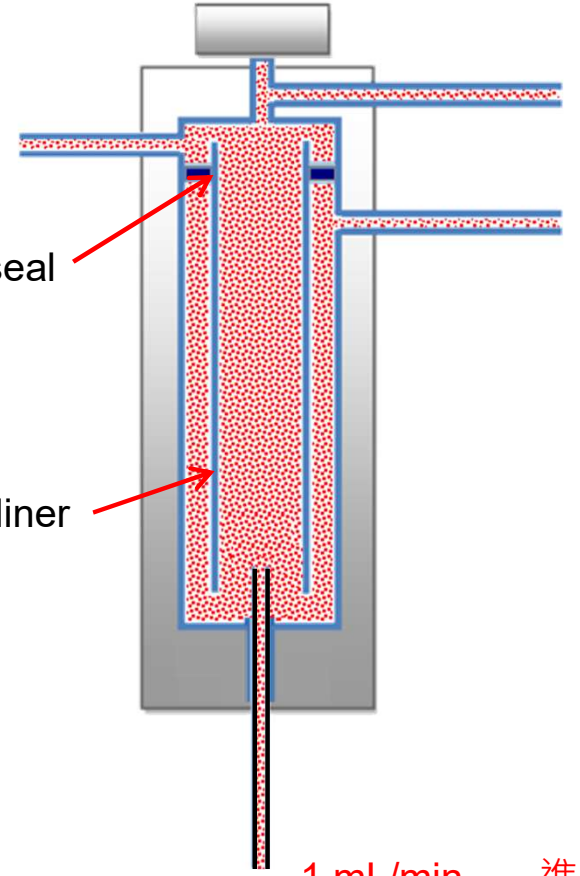
- 分流比可用 50:1、 100:1 、 30:1….來表示

- 例子:
  - 分流控制閥流速 = 100 mL/min, 管柱流速 = 2 mL/min
  - 分流比 = 50:1 或是大約 2% 的樣品進入到管柱中

典型的分流進樣

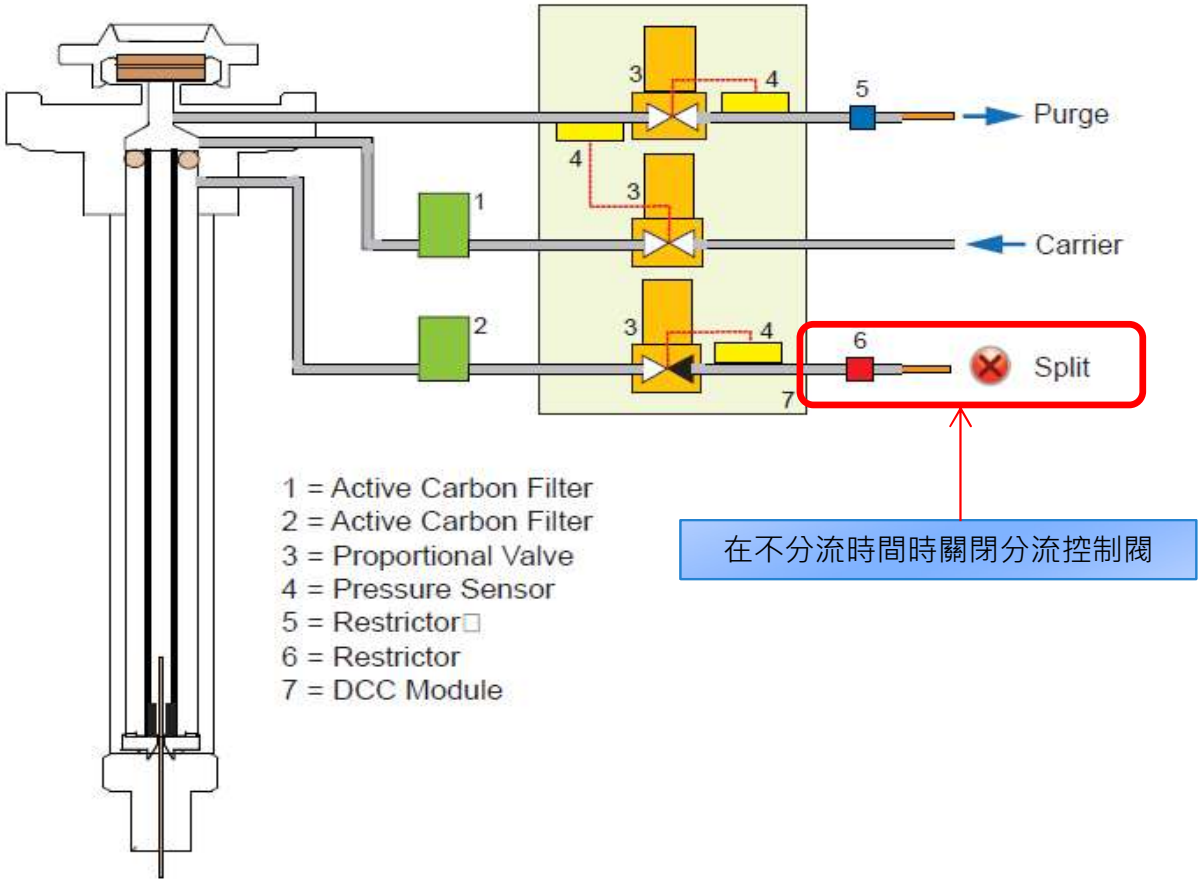
Helium

56 mL/min Carrier inlet

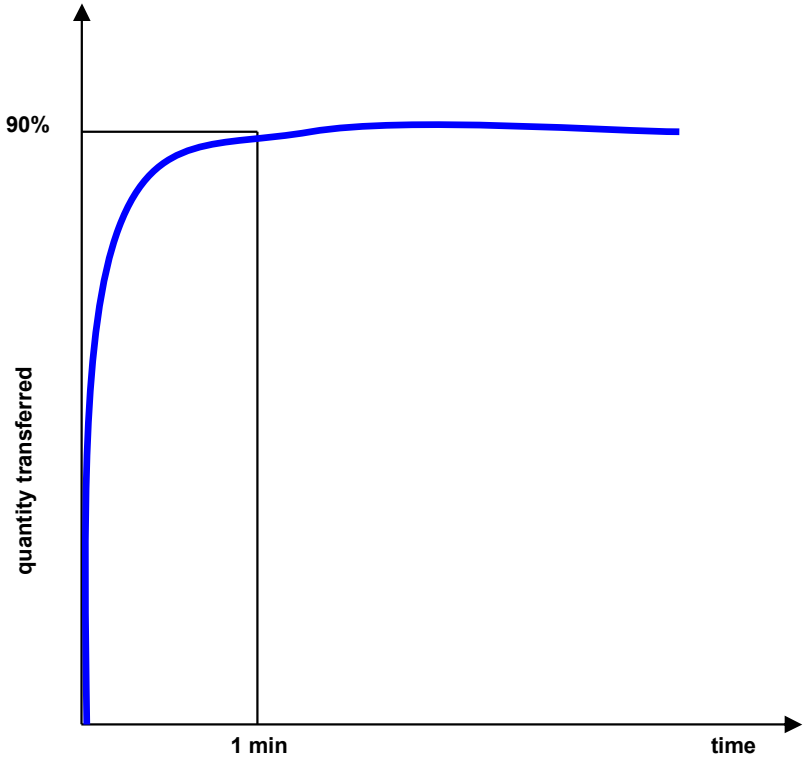


進樣器中的壓力控制閥決定進入管柱中的流量

不分流的進樣模式



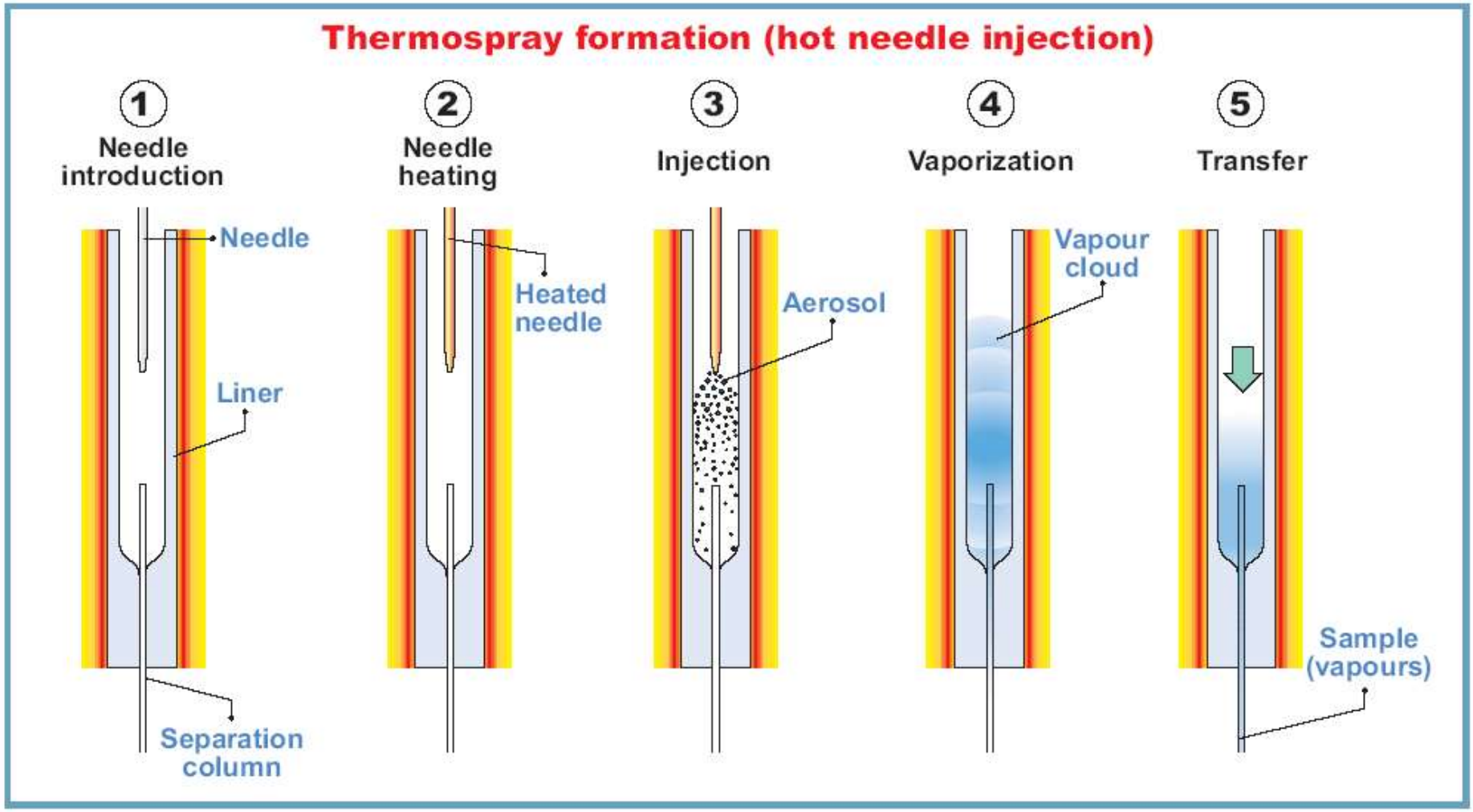
樣品轉移進入到層析管柱



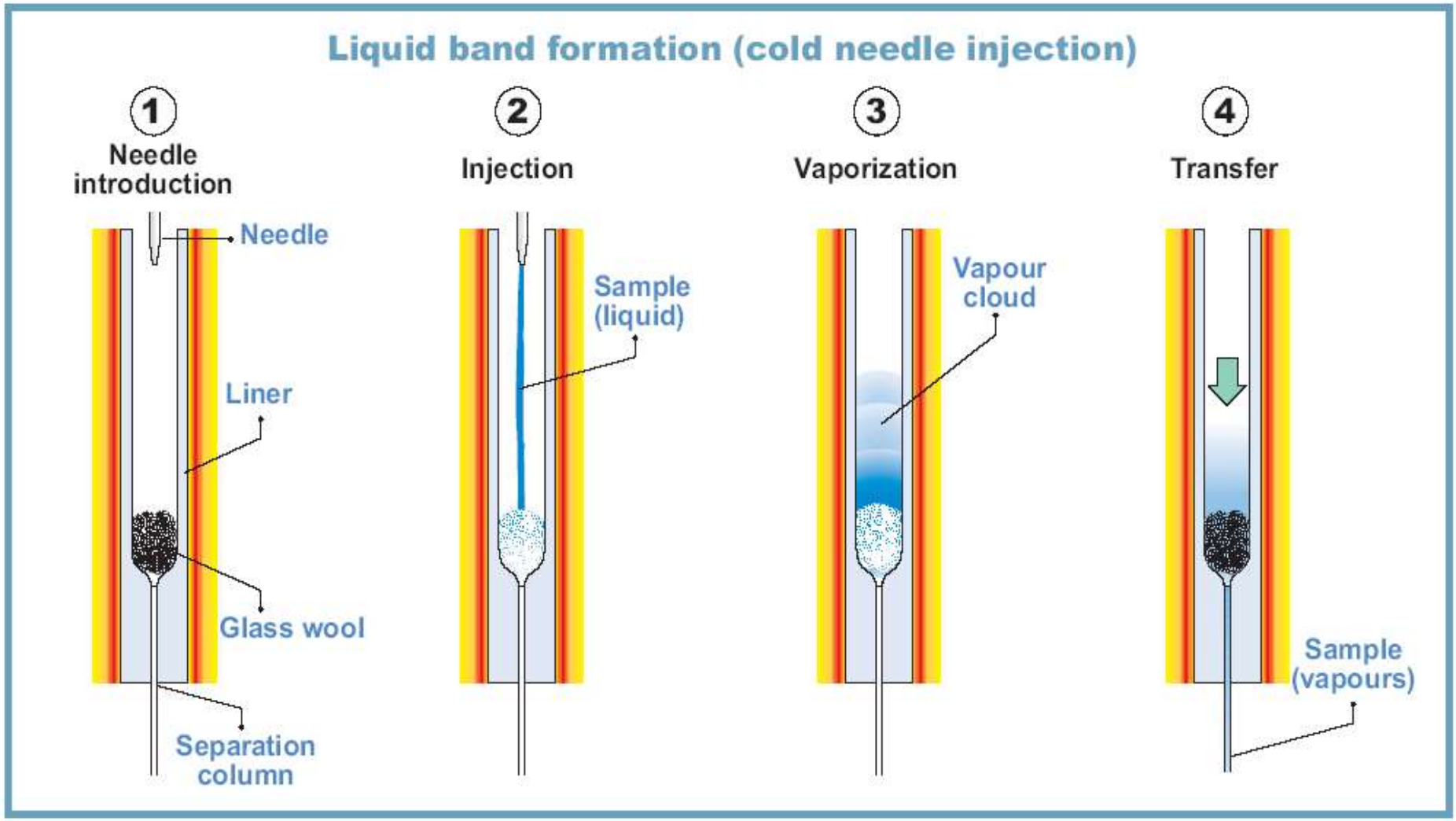
- 需要依據注射量來進行計算
- 計算的因素有;
  - 使用的溶劑種類
  - 注射量(體積)
  - 注射器溫度
  - 管柱可乘載的壓力
- 建議不分流時間需兩倍的汽化管體積

圖中所示的 90% 轉移應該以兩倍 於載氣襯裡體積的掃過完成。 例如，襯管體積 1.5 ml，柱流速 1ml/min，不分流時間 = 3.0 min：

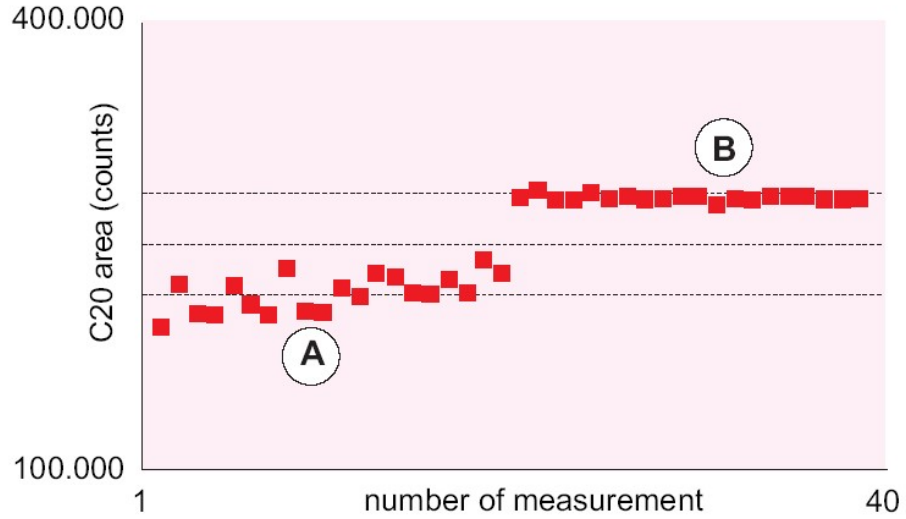
注射模式 / 熱噴灑 (Thermospray formation)



注射模式 / 液體層 (Liquid band formation)



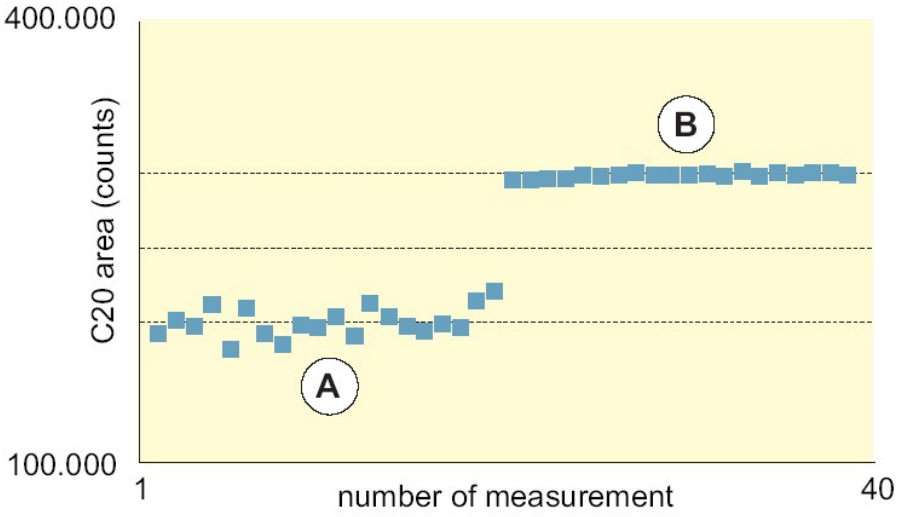
如何最佳化注射口參數



熱噴灑

(A) 注射針加熱時間太短 (0.1 s)

(B) 注射針加熱時間正確 (5s)



液體層

(A) 無石英棉

(B) 有石英棉

哪種注射方式較佳

熱噴灑:

1. 提供更溫和且可靠的樣品氣化方式
2. 適用熱不穩定且易被吸附的化合物

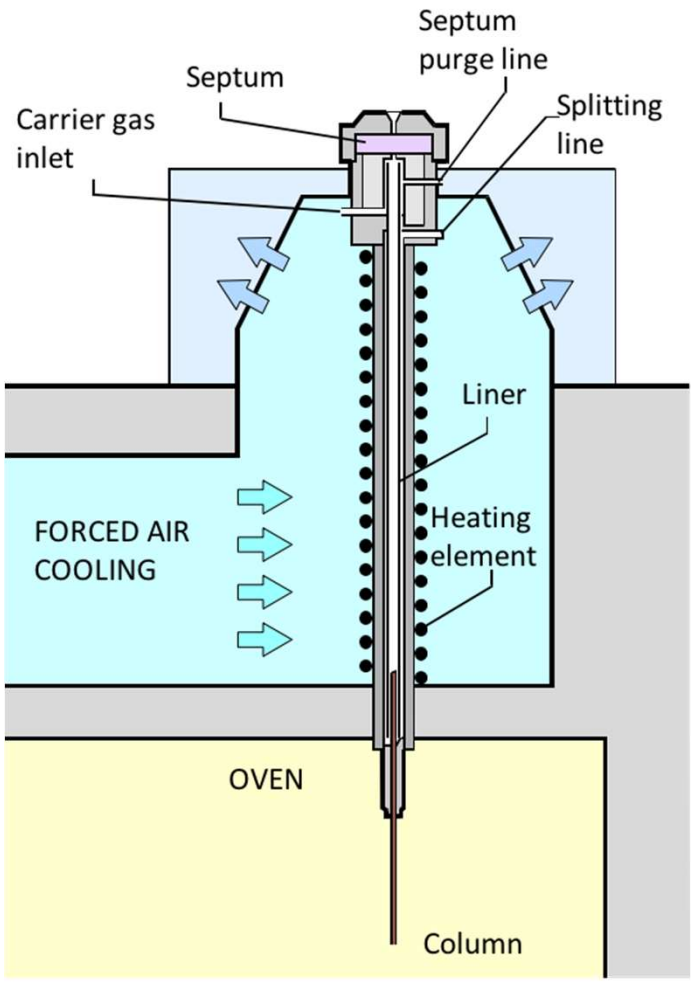
液體層:

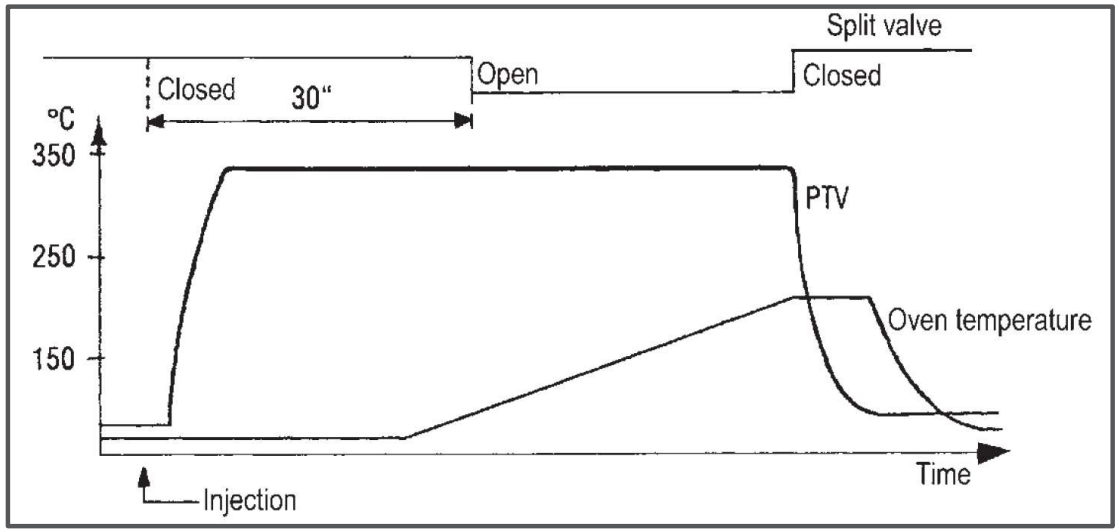
1. 適用非常骯髒的樣品，特別是在使用內徑較小的管柱上
2. 注射少量的高濃度樣品

- 選擇其中一種，並進行最佳化
- 避免交替使用

進樣模式2: 程序升溫注射口 (PTV)

- **Programmable Temperature Vaporizing injector**

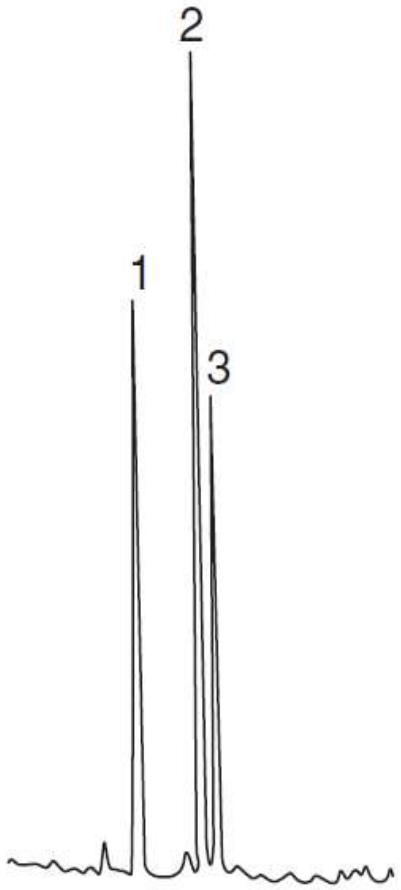




- 最佳化注射口溫度
- 較低的質量歧視效應 (mass discrimination)
- 避免分析物的熱分解 (thermal decomposition)
- 可提升樣品進樣體積

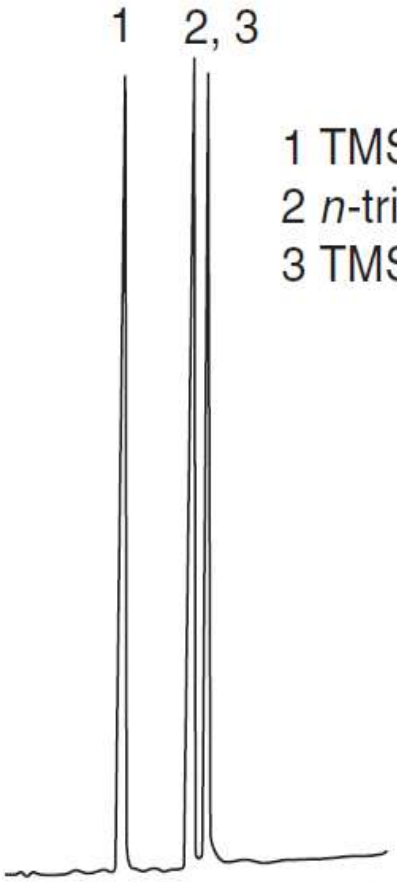
兩種注射口的差異

分流/不分流注射口



Isothermal injection 280°

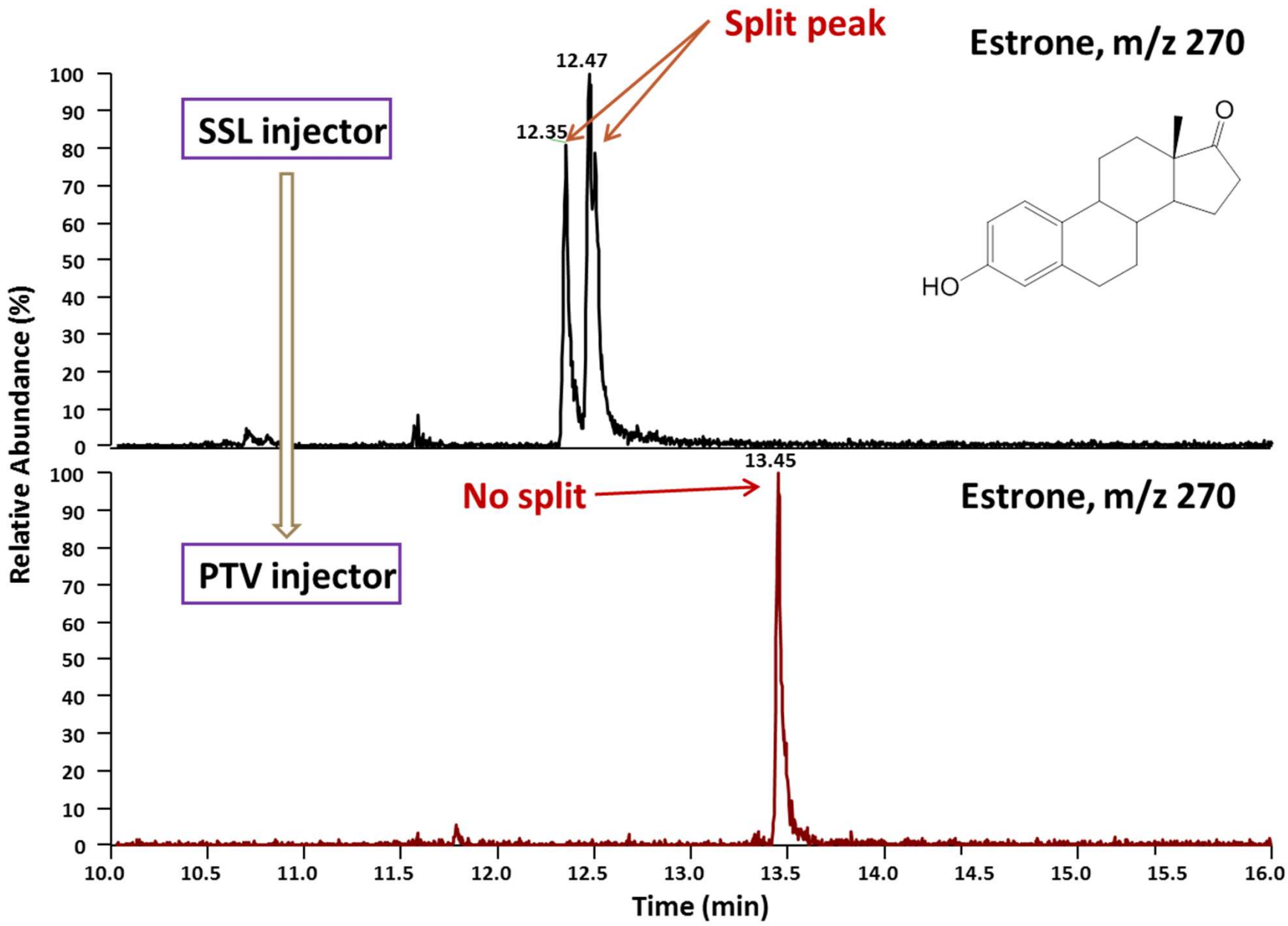
程序升溫注射口



1 TMS ester of *n*-tetracosanoic acid
2 *n*-triacontane
3 TMS ester of *n*-hexacosanoic acid

Injector programmed 130°, 100° per minute to 280°

兩種注射口的差異



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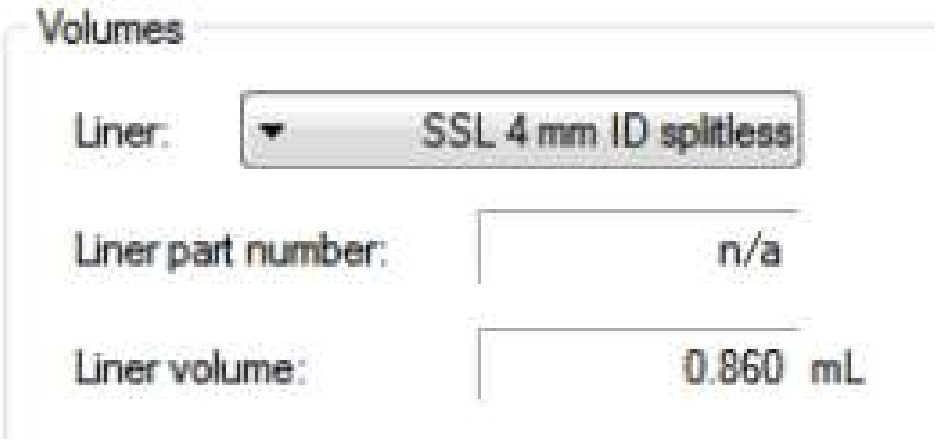
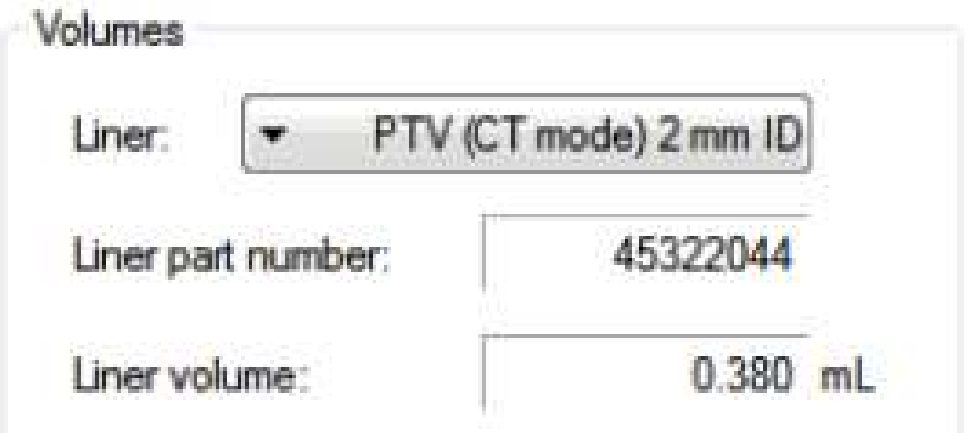
兩種注射口的差異





- ½ Liner Volume

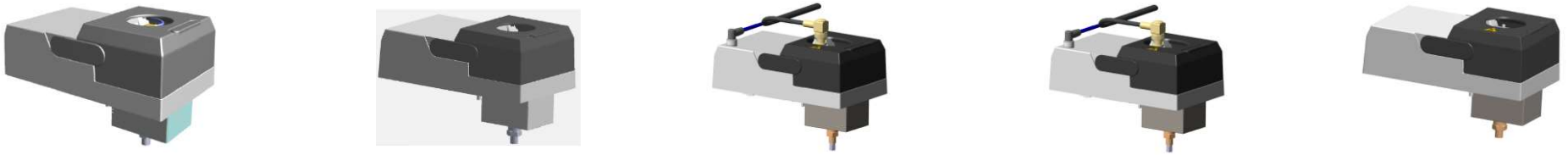
注射口比較

優: +　　劣: -

	SSL	PTV
無質量歧視效應 (Discrimination free)	+	++
對較髒的樣品比較耐用 (Resistance to “dirty” matrices)	++	+++
熱不穩定分析物 (Compatibility with thermolabile compounds)	-	+
活性分析物 (Compatibility with active compounds)	++	++
不稀釋樣品 (Compatibility with undilutable samples)	++	+++
使用彈性 (Amenability for different samples)	+	+++
大體積進樣 (Large sample volume capabilities)	+	++
使用簡便性 (Ease of use @ system set up)	+++	+
快速GC適用性 (Compatibility with fast GC)	++	+++

GC偵測器總覽

FID ECD NPD FPD TCD



- ISQ
- TSQ8000EVO
- Orbitrap

MS GC

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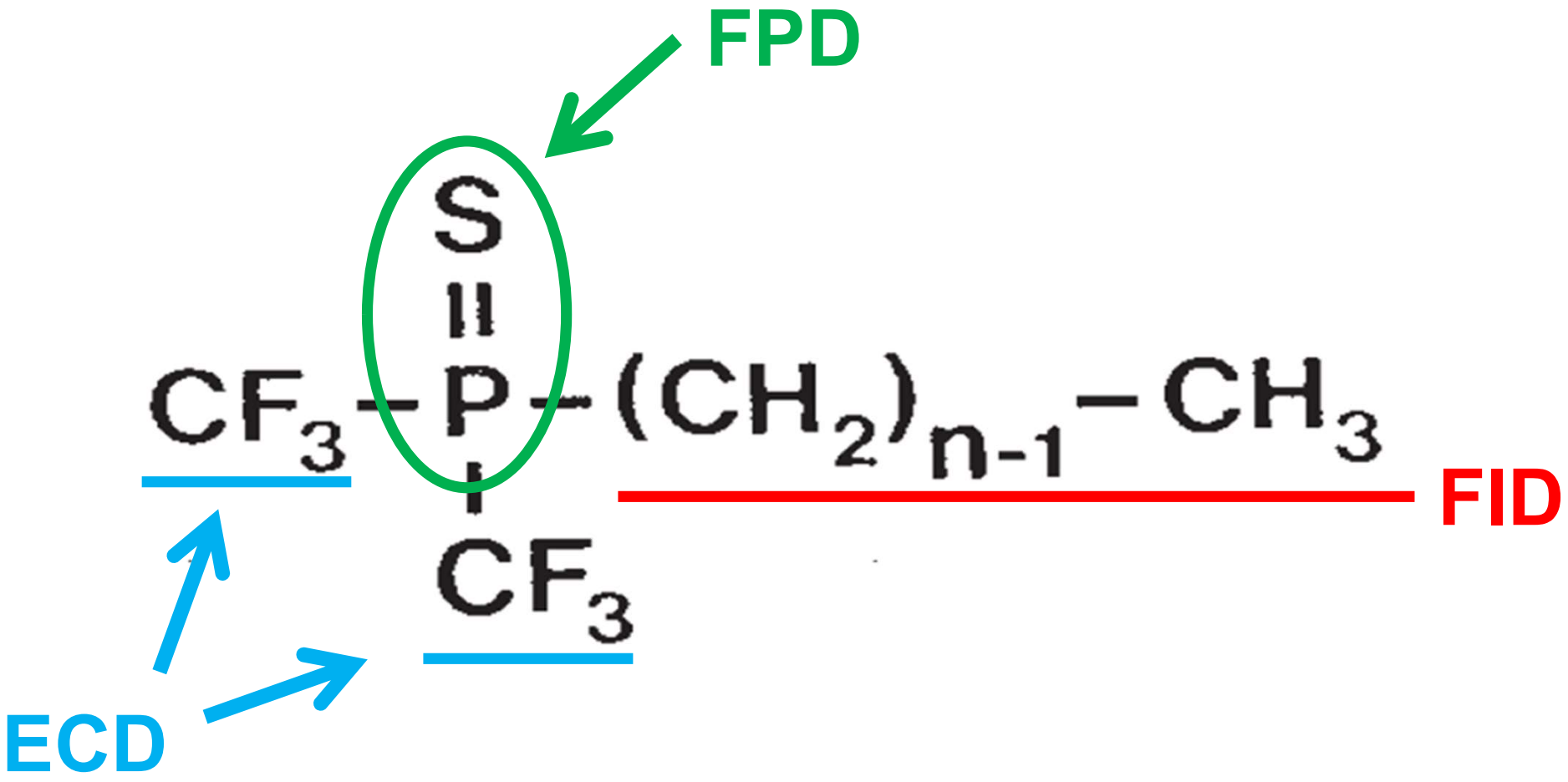
偵測器靈敏度

Table 1

Name	Acronym	Type of Response	Detected Species	Response Characteristic	Destructive	LOD	Dynamic Range	Linear Range	Selectivity
Flame Ionization Detector	FID	universal to C	carbon	mass	Y	10^{-12} g C/sec	10^{7}	10^{7}	na
Thermal Conductivity Detector	TCD	universal	thermal conductivity	concentration	N	10^{-9} g/mL	10^{5}	$<10^{5}$	na
Nitrogen/Phosphrus Detector, Thermionic Detector	NPD	selective	nitrogen or phosphorus	mass	Y	10^{-12} g N/sec	10^{5}	10^{5}	25000 N vs C 75000 P vs C
Flame Photometric Detector	FPD	selective	phosphorus or sulfur	mass	Y	10^{-13} g P/sec 10^{-12} g S/sec	10^{4} 10^{3}	10^{4} for P non-linear S	10^{6} P vs C 10^{6} S vs C
Electron Capture Detector	ECD	selective	electronegative groups such as halogens, oxygen containing-groups	concentration	N	10^{-14} g/mL	10^{5}	10^{4}	up to 10^{6} vs C depending on type and number of halogens
Chemiluminescence Detector	SCD NCD	selective	sulfur nitrogen	mass	Y	10^{-12} g S/sec 10^{-12} g N/sec	10^{5} 10^{5}	$>10^{4}$ $>10^{4}$	10^{7} S vs C
Photo-ionization Detector	PID	selective	ions of photo dissociated compounds	mass	N	10^{-12} g/sec	10^{7}	10^{6}	∞ against compounds with ionization potentials higher than source energy
Atomic Emission Detector	AED	both	atomic emission	mass	Y	10^{-12} to 10^{-10} g/sec	$10^{3} - 10^{4}$	$10^{3} - 10^{4}$	$10^{3} - 10^{4}$ vs C
Mass Selective Detector, Mass Spectrometer	MSD	both	ionized molecular fragments	mass	Y	10^{-13} g	10^{6}	10^{6}	∞ for ions outside mass resolution window
Inductively Coupled Plasma Mass Spectrometer	ICP-MS	both (universal if measuring carbon)	ionized atoms	mass	Y	10^{-14} g/sec	10^{6}	10^{6}	∞ outside mass resolution window
Electrolytic Conductivity Detector	ELCD	selective	halogens	mass	Y	10^{-15} g/sec	10^{6}	10^{5}	$10^{5} - 10^{6}$ vs C
Infrared Detector	IR	both	molecular vibrations	concentration	N	10^{-9} g/mL	10^{5}	10^{4}	$10^{2} - 10^{4}$ depending on functional group

- **TCD, PDD**



偵測器選擇

Universal

They respond to everything eluting from the column

- TCD
- PDD
- FID

Selective

Element selective, Structure selective, functional group selective

- FID
- ECD
- PID
- PDD

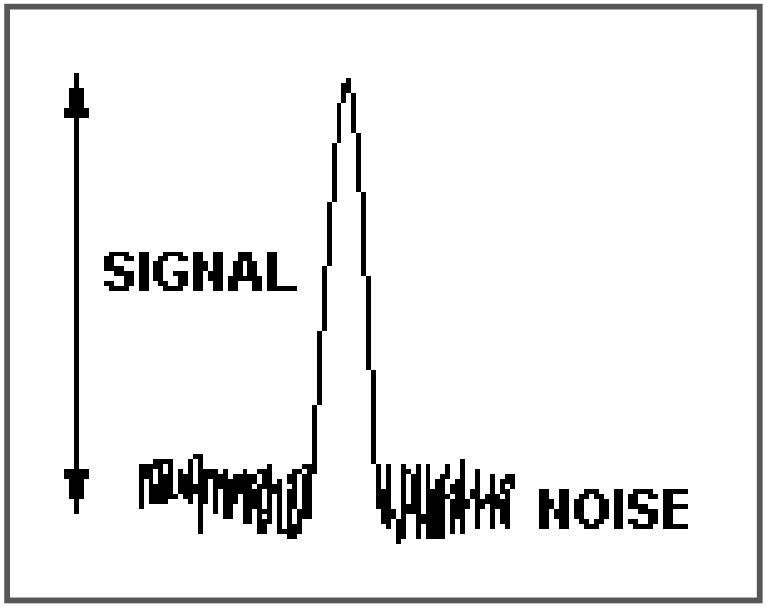
Specific

They are so selective to distinguish particular structures or elements

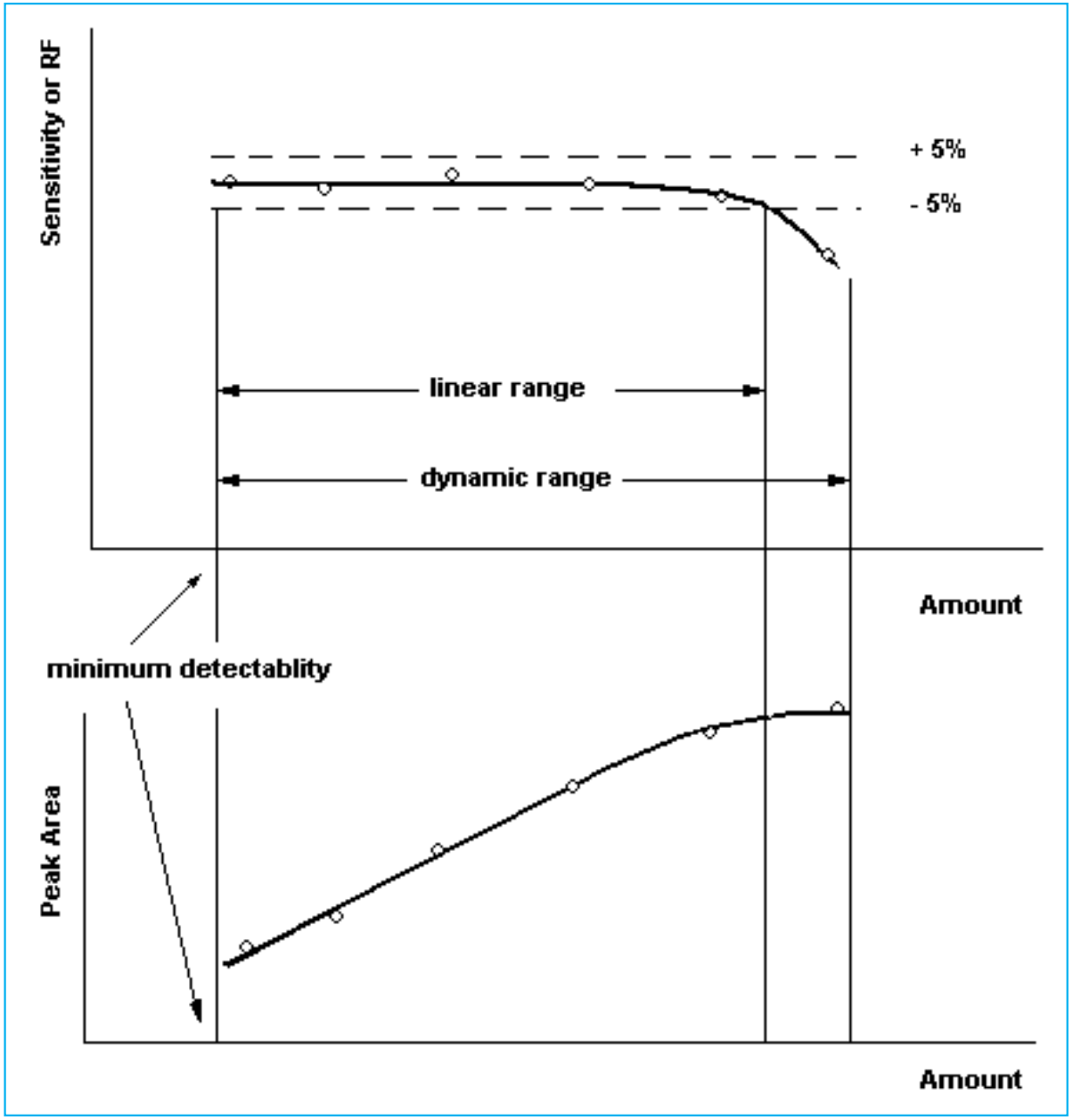
- NPD
- FPD
- PFPD

偵測器效能特性

1. **Sensitivity**
   - Sample amount convert to electronic signal
2. **Selectivity**
   - Sensitivity of compounds in matrix interference
3. **Dynamic Range**
   - Range of concentration could detect
4. **Minimum Detectability**
   - S/N = 3
5. **Noise**



偵測器效能特性/ 線性範圍與動態範圍



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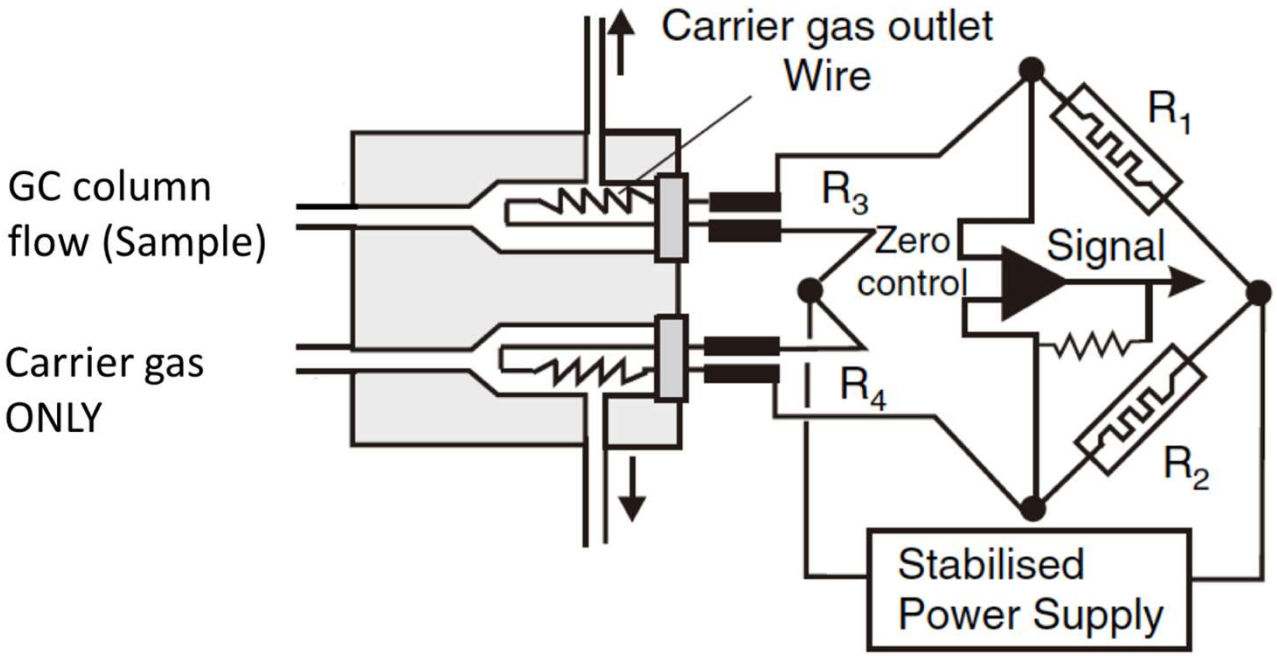
TCD / Thermal Conductivity Detector

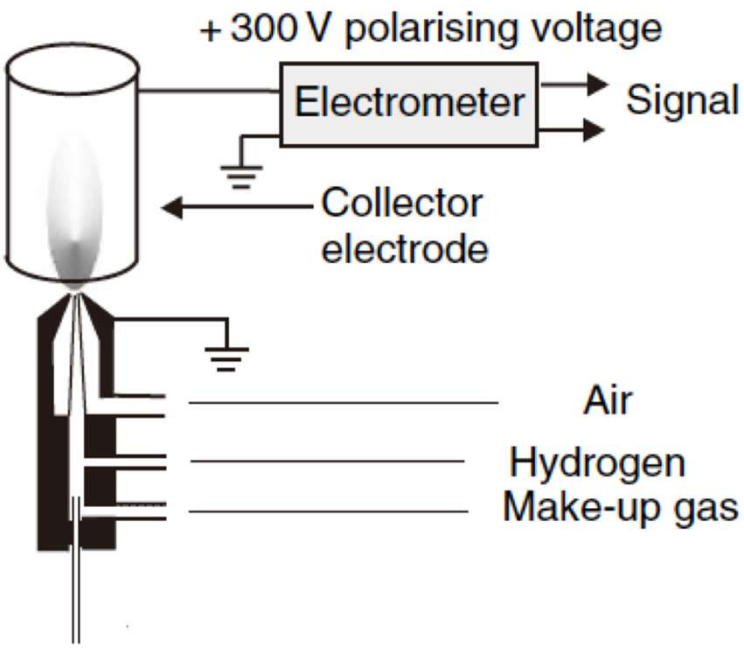


- ✓ **Universal**
- ✓ **Simple**
- ✓ **For gas analysis**

- ***Thermal conductivity*** of gas mixtures
- Both thermistors are located within the path of the carrier gas
- One is flushed by the carrier gas evolving the column, while the other is flushed by a part of the carrier gas entering the injector.

Chemical Analysis: Modern Instrumentation Methods and Techniques (2nd Ed.), Wiley
Handbook of GC/MS: Fundamentals and and Applications (2nd Ed.), Wiley

FID / Flame Ionization Detector



Reactions in FID:

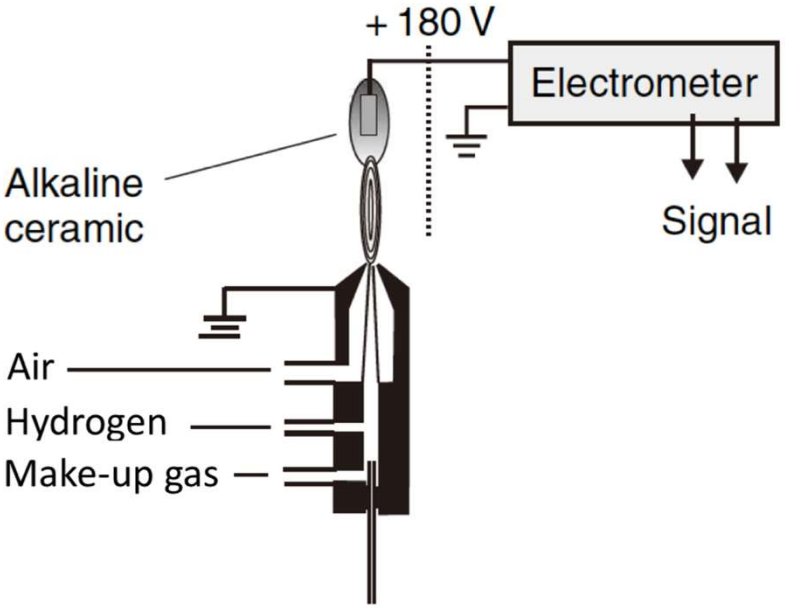
Pyrolysis

$$CH_2^o + OH^* \rightarrow CH_3O^+ + e^-$$
$$CH^o + OH^* \rightarrow CH_2O^+ + e^-$$
$$CH^o + O_2^* \rightarrow CHO_2^+ + e^-$$
$$C^o + OH^* \rightarrow CHO^+ + e^-$$

- FID responds to ***all organic compounds*** with favorable sensitivity
- FID response is ***proportional to the number of carbon atoms***
- The detector response is not affected by modest changes in flow, pressure, or temperature
- FID does ***not respond to common carrier gas impurities*** such as CO_2 and water

Chemical Analysis: Modern Instrumentation Methods and Techniques (2nd Ed.), Wiley
Handbook of GC/MS: Fundamentals and and Applications (2nd Ed.), Wiley

NPD / Nitrogen Phosphorous Detector



Reactions for "*P-containing substances*"

$$[O{=}P]^{\bullet} + \text{Alkaline}^{*} \rightarrow [O{=}P]^{-} + [\text{Alkaline}]^{+}$$

$$[O{=}P]^{-} + OH^{\circ} \rightarrow HPO_2 + e^{-}$$

Reactions for "*CNC-containing substances*"

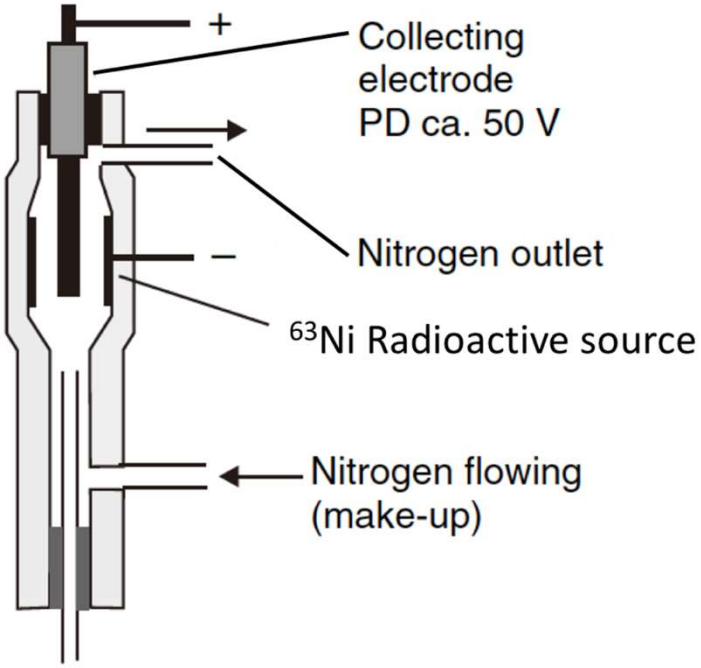
$$C{\equiv}N^{\circ} + \text{Alkaline}^{*} \rightarrow CN^{-} + [\text{Alkaline}]^{+}$$

$$CN^{-} + H^{\circ} \rightarrow HCN + e^{-}$$

- Nitrogen-phosphorous detector (NPD) is a **modified FID**
- Phosphorus-containing substances are first converted in the flame into phosphorus oxides with an uneven number of electrons.
- ***C-N structure*** must already be present in the molecule

Chemical Analysis: Modern Instrumentation Methods and Techniques (2nd Ed.), Wiley
Handbook of GC/MS: Fundamentals and and Applications (2nd Ed.), Wiley

ECD / Electron Capture Detector



Reactions in ECD

$$\text{Carrier gas (CG)} \xrightarrow{\beta} CG^+ + e^-$$

$$M + e^- \rightarrow M^-$$

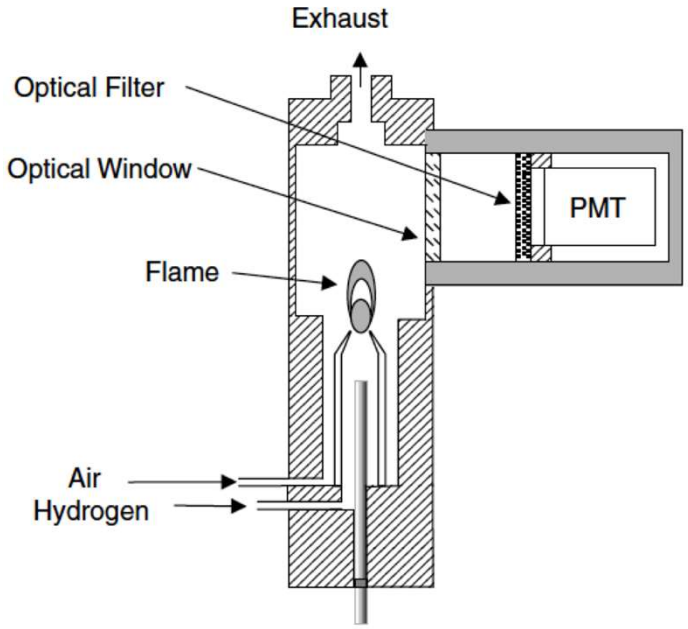
!! **Radioactive radiator**

!! **Handling authorisation is necessary**

- The ECD reacts with ***all electronegative functional groups (-F, -Cl, -Br, -OCH_3, -NO_2)***
- ***All hydrocarbons*** (generally the matrix) ***remain transparent***, although present.
- Sensitive to misuse Mobile only under limited conditions
- ***Ideal for trace analysis***

Chemical Analysis: Modern Instrumentation Methods and Techniques (2nd Ed.), Wiley
Handbook of GC/MS: Fundamentals and and Applications (2nd Ed.), Wiley

FPD / Flame Photometric Detector

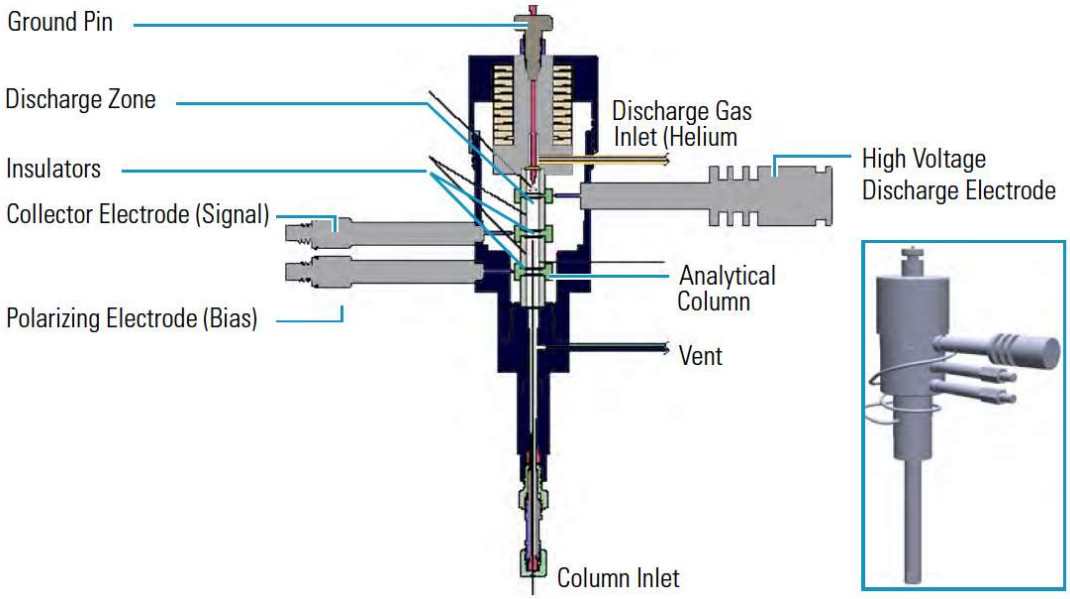


✓ **Dual FPD is optional to analyze P- and S-containing substances simultaneously**

- In a hydrogen-rich flame ***P-*** and ***S-***containing radicals are in an excited transition state.
- On passing to the ground state a characteristic ***band spectrum is emitted*** (S: 394 nm, P: 526 nm).
- The flame emissions initiated by the eluting analytes (chemiluminescence) are determined using an ***optical filter and amplified by a photomultiplier***

Modern Practice of Gas Chromatography (4th Ed.), Wiley
Handbook of GC/MS: Fundamentals and and Applications (2nd Ed.), Wiley

PDD / Pulsed Discharge Detector



Reactions in PDD:

$$He_2(A^1\Sigma_u) \rightarrow 2He(1^1S_0)$$

IE: 13.5 to 17.5 eV

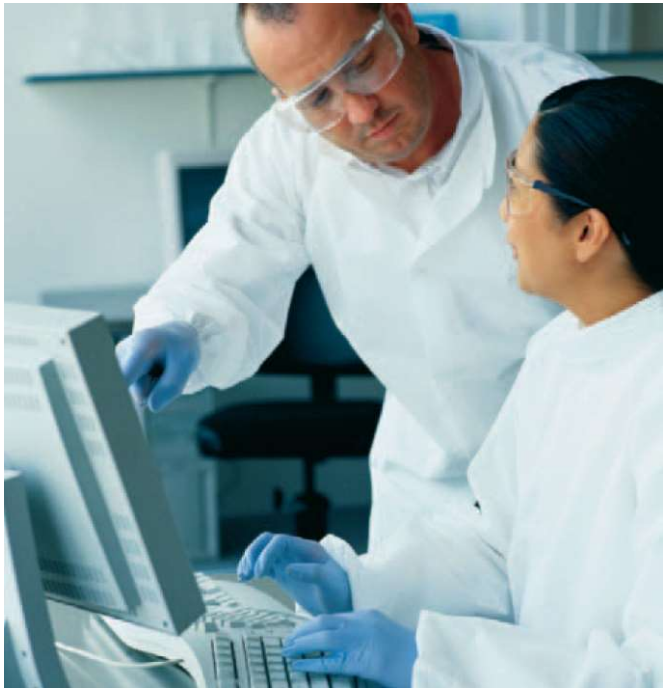
!! Even highest quality carrier gas may contain some water vapor and fixed gas impurities, a helium purifier is included as part of the detector system.

- An ***universa***l and ***highly sensitive non-radioactive*** and ***non-destructive*** detector
- This detector does ***not use radioactive*** sources
- The ***response to organic compounds*** in the ***low pg range***
- The ***response to fixed gases*** in the low ***ppb*** range

D.S. Forsyth, Pulsed discharge detector: theory and applications, *J. Chromatogr. A*, 2004, 63
Handbook of GC/MS: Fundamentals and and Applications (2nd Ed.), Wiley

氣相層析與氣相層析質譜故障排除
(Troubleshooting for GC and GC-MS)

- 一步一步來 (Steps by steps)
- 層析或質譜的問題



第一步 / 找出與確認問題點所發生的點

GC + MS

- 靈敏度 (Sensitivity)
- 選擇性 (Selectivity)

- 波峰形狀 (Peak shape)
- 解析度 (Resolution)
- 有無波峰
- 去活化效果 (Deactivation)

- 靈敏度 (Sensitivity)
- 選擇性 (Selectivity)

- 以原理方式思考
- 汙染 (Contamination)
- 漏氣
- 質量偏移

確認問題點

- 定義問題
- 確認樣品與保養維護紀錄來確認數據的品質與趨勢
- 用邏輯思考來過濾問題點

最明顯跟容易的地方開始

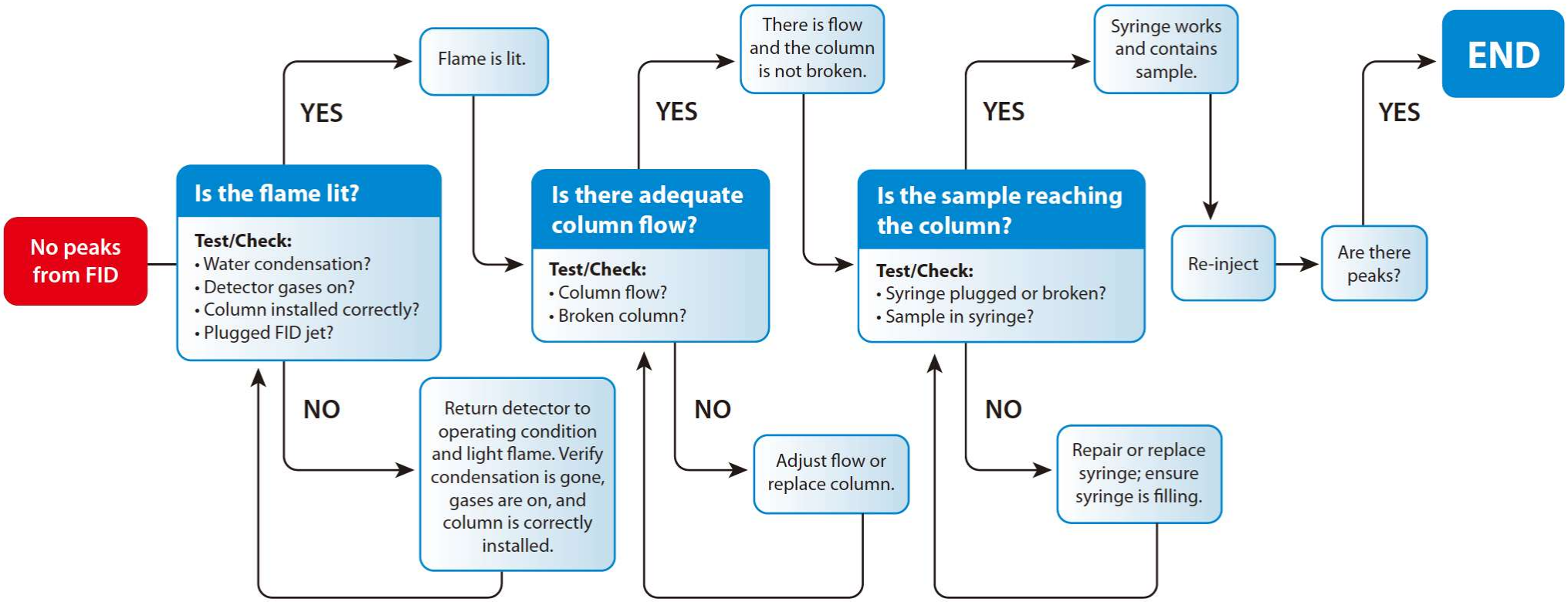
- 電源開關
- 機器間的電源線連接
- 機器間的訊號線連接
- 氣體純度
- 氣體流速
- 溫度設定
- 注射針狀況
- 樣品製備
- 分析方法與參數設定

記錄做過的嘗試

- 請紀錄所有嘗試過的步驟與其結果 (獲取經驗)
- 分析**自定義的測試標準品**並跟之前的數據做比較

以氣相層析火焰離子偵測器 (GC-FID)為例: 沒有波峰出現?

問題點　　第一次嘗試:
火焰是否開啟　　第二次嘗試:
氣體流速是否正確　　第三次嘗試:
樣品是否有真的進去GC　　解決!!



邏輯 & 紀錄

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從GC端開始

- **不論您用哪種注射口**

  90% 的問題來自注射口系統!!

- 注射針, 接環 (ferrule), 墊片(septum), 襯管(liner), 分析管住…
- 汙染, 破損或堵塞

請定期確認與維護

從GC端開始 / 自動進樣器 (autosampler)

- 注射針部分，如: 殘留記憶效應(carryover effect), 樣品未被吸取
- 樣品瓶上的墊片被過度使用(Overusing)
- 樣品未被選擇

注射針 / 手動注射與自動注射方式

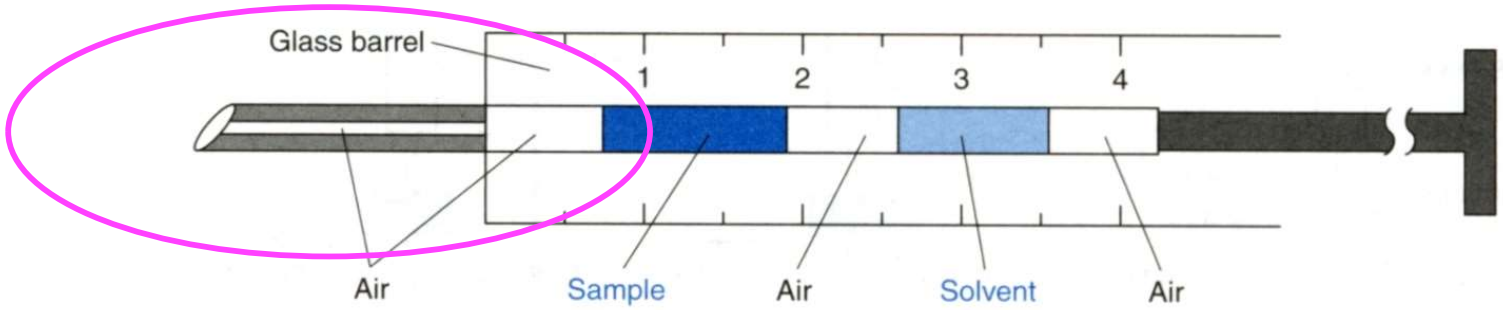
- 好的注射針使用技巧可以達到令人滿意的GC-MS分析工作
  - 彎曲(Bend)與堵塞(blocked)是常見的問題
  - 注射針的內部與外部皆有可能為主要的污染來源

- 使用適合的有機溶劑來清洗注射針，如丙酮 (acetone)與甲醇 (methanol)

手動注射方式 / 三明治注射法 (Sandwich injection)

- 增加再現性 (Reproducibility)

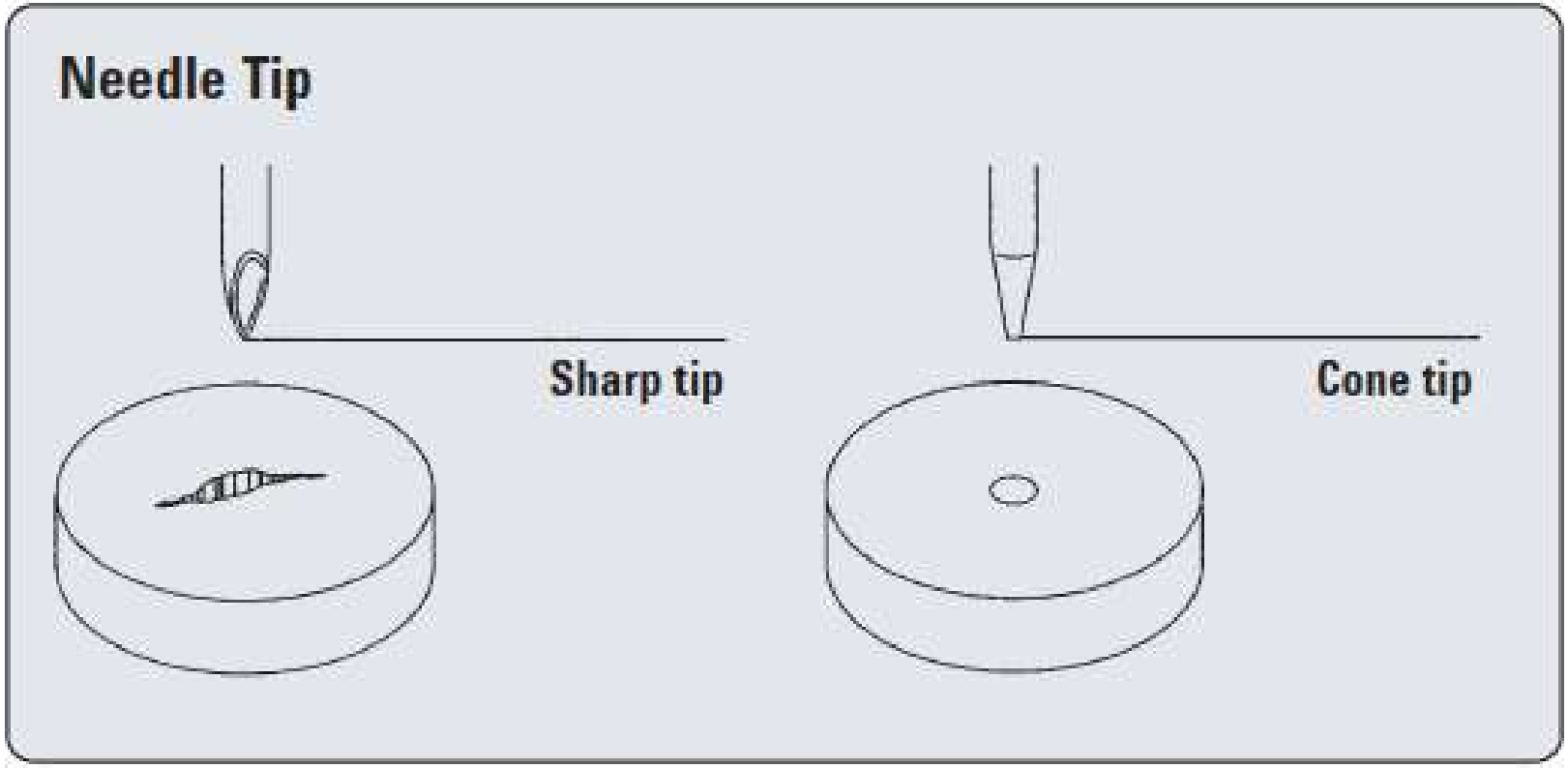
樣品的注射體積為 0.1 - 2 µL



利用空氣與溶劑來分隔樣品

- 抽取空氣來間隔樣品的目的為避免大部分的揮發性分析物在注射完成之前會逸散的問題
- 溶劑是用來將樣品完全導入注射口，但會跟樣品之間用空氣來避免混和
- 最後一段的空氣是用來將溶劑完全導入注射口

注射針 / 注射針前端 (needle tip)



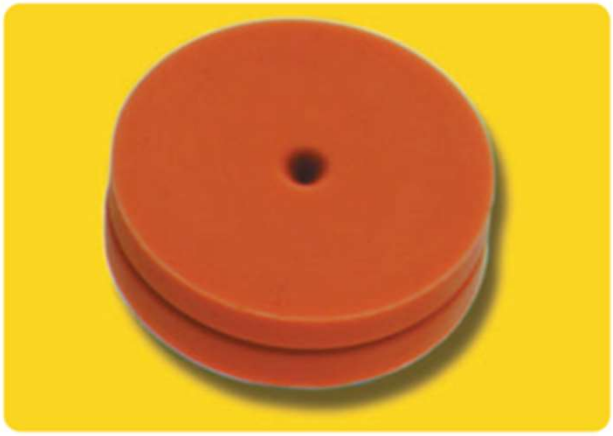
斜口的尖頭注射針會導致

(1) 撕裂墊片而產生漏氣

(2) 將樣品殘留在墊片上

(3) 在層析圖上有很大且拖尾的溶劑波峰

墊片特性



BTO

Long Life

Advanced Green

墊片種類	流失現象 (bleed)	使用壽命	承受溫度
BTO	✓✓✓	✓	to 400 °C
Long Life	✓	✓✓✓	To 350 °C
Advanced Green	✓✓	✓✓	To 350 °C

- BTO: Bleed and Temperature Optimized
- ✓✓✓ = best ; ✓✓ = very good ; ✓ = good

墊片的更換時機

1. 建議至少應在注射樣品**200次** (50 – 100次為佳)更換墊片，或是發現問題與墊片有關聯時
2. 樣品污染與環氧烷(siloxanes)訊號(GC-MS)

如何更換墊片 (不需要將GC-MS關機!!!)

1. 降低注射口溫度 (TRACE 1300/1310不需要此步驟)
2. 關閉氣體流速
3. 換上新的墊片

❖ **注意事項:**

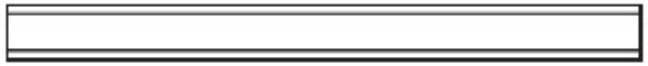
請勿過度鎖緊注射口的上蓋，會更加容易造成墊片的受損與影響分析效能

襯管(Liner)的選擇 / 分流、不分流　(TRACE1300系列SSL注射口)

Split

A

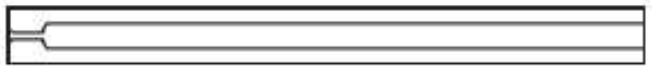
B



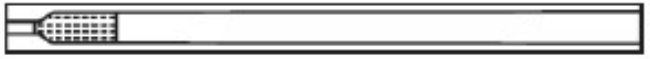
- A: 含有石英棉，增加樣品均勻程度 (No: **453A2265**)
- B: 襯管A的完全開口型 (No: **453A1295**)

Splitless

C



D



- C: 亦可用於不分流 (No: **453A1345**)
- D: 含有石英棉 (No: **453A1925**)

Splitless (ID 1.2 mm)

E

- E: 表面積小，適用於不穩定物質 (No: **453A1335**)

襯管(Liner)的選擇 / 分流、不分流

1. 襯管需**定期**更換:

   - **取決於樣品注射次數或樣品的特性**

2. **如何清潔襯管**

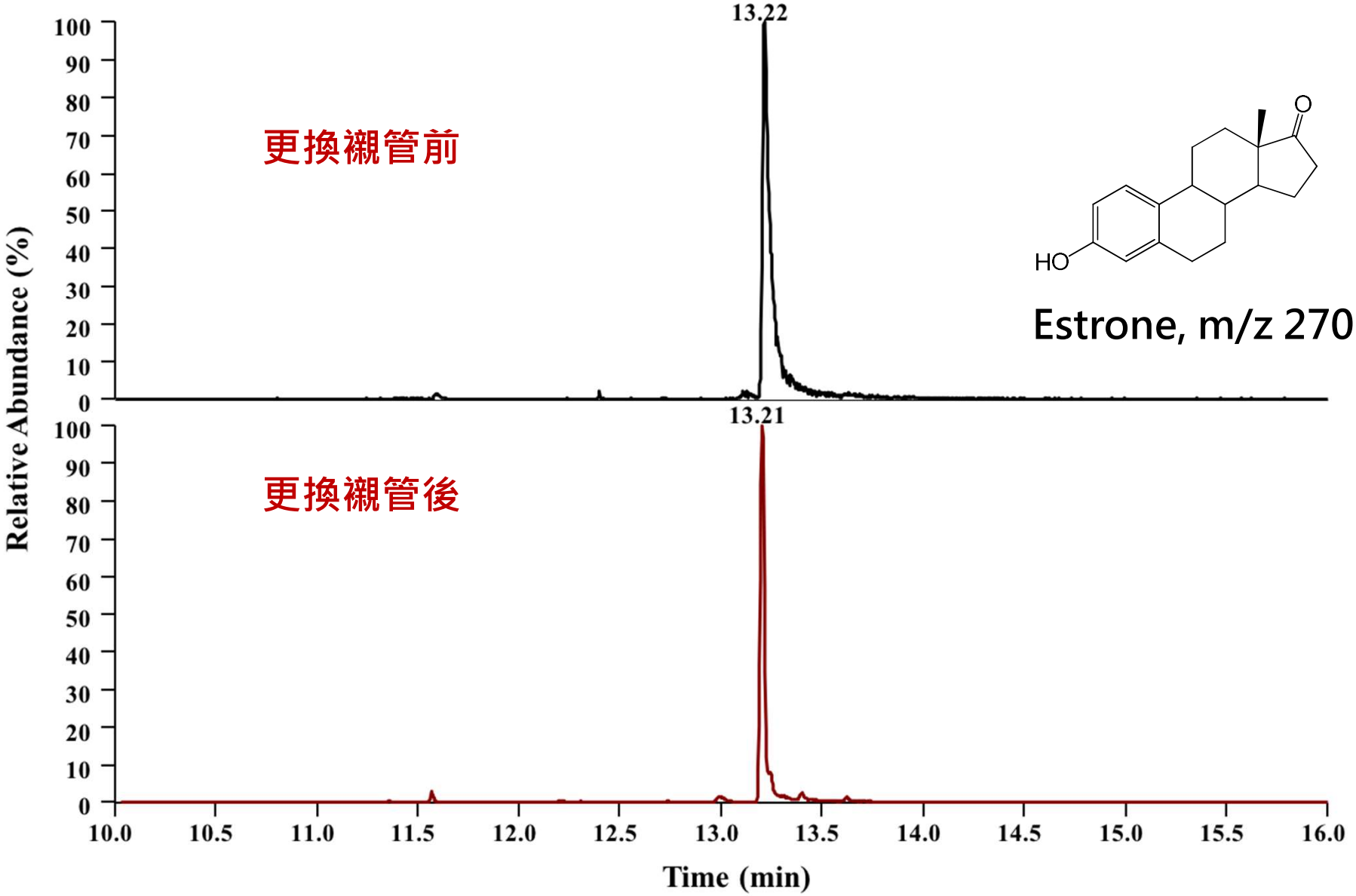
   – 使用乾淨的壓縮氣體或刷子先移除顆粒狀汙染物

   – 使用甲醇/丙酮(1:1)的混合溶劑進行**超音波震盪**30 min

   – 如有必要可使用清潔劑清潔

3. **去活化(矽烷化)**可避免襯管吸附活性分析物

襯管的更換 / 改善拖尾現象



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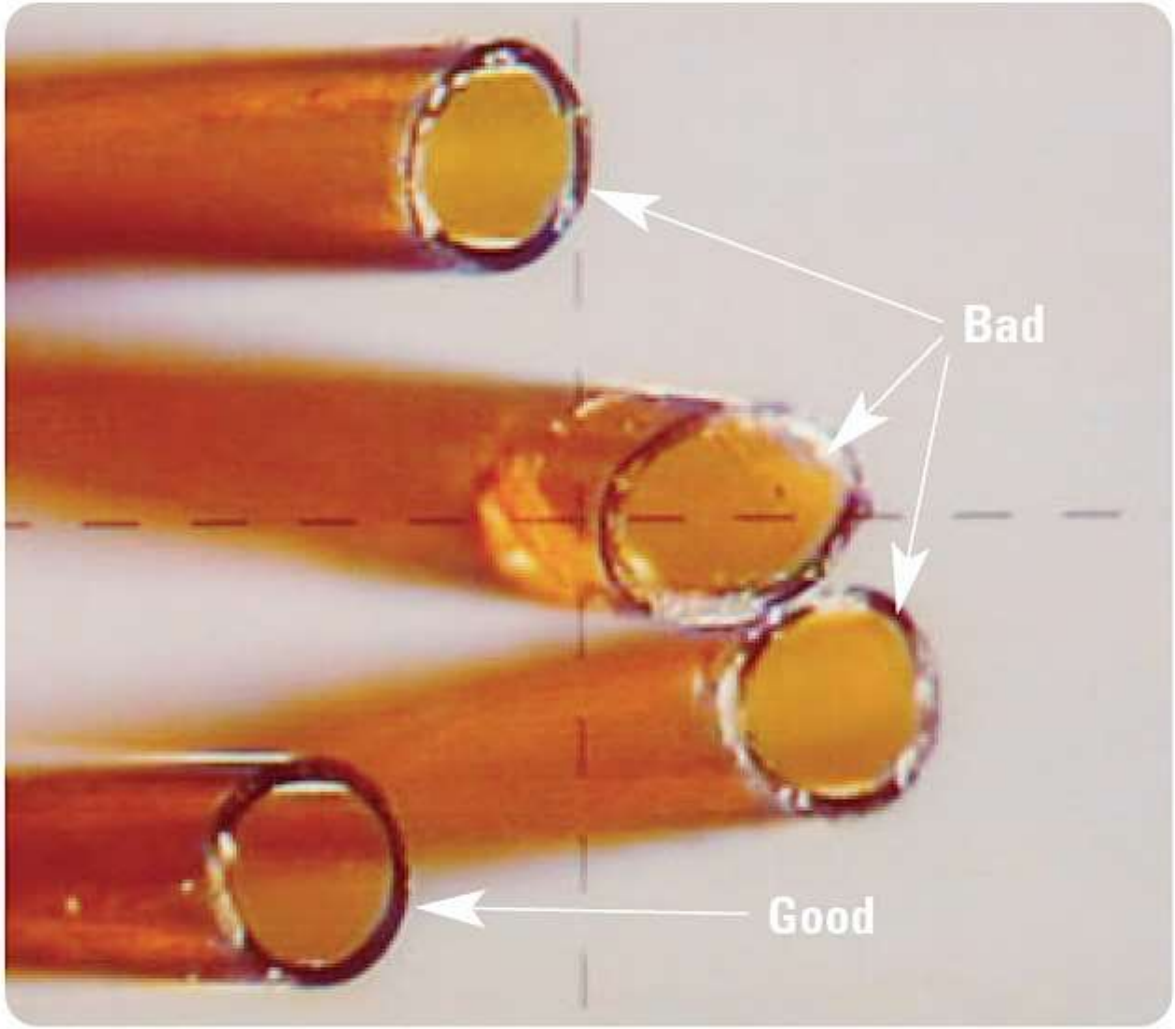
管柱裁切後的切面



仔細確認~

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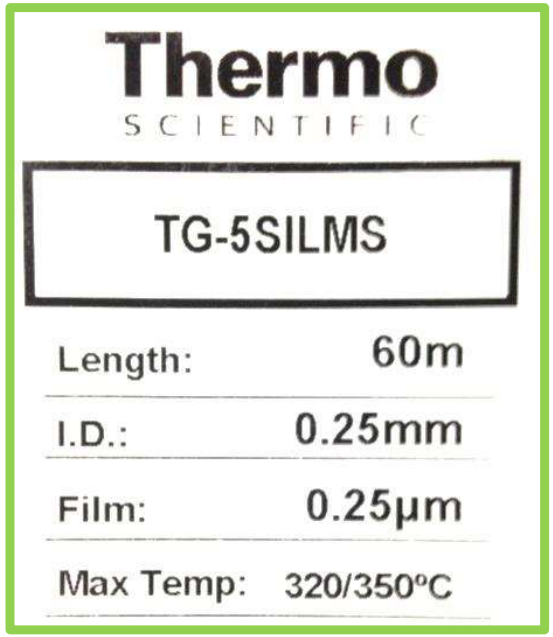
管柱 / 效能下降

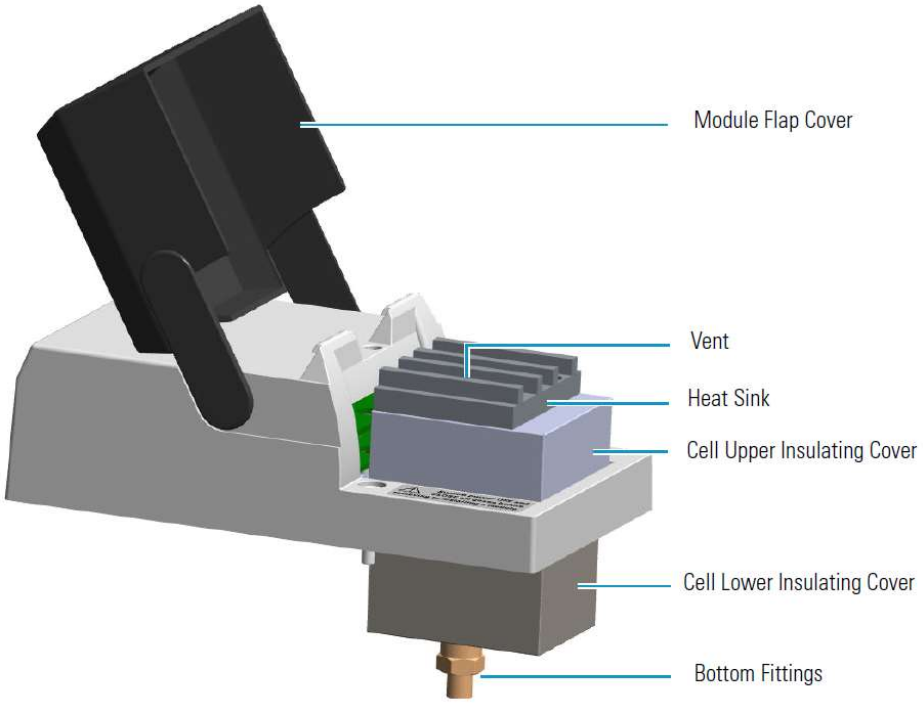
	如何避免
損壞(Column Breakage)	• 避免刮傷與擦傷 • 避免過度彎曲管柱
熱損害(Thermal Damage)	• 別超過管柱的耐溫上限: - 恆溫上限(isothermal limit) : 可維持無限長的時間 (ex: 120 min) - 變溫上限(Programmed limit) : 可使用的最高溫度 (不建議使用到這麼高溫) • 可定義GC的最高使用溫度是 ≤ 管柱的溫度上限



偵測器的保養與確認

TCD

- **一般而言，不需要特殊的保養**
- **避免Filament的汙染與損壞**
- **必須送回工廠進行Filament的更換**



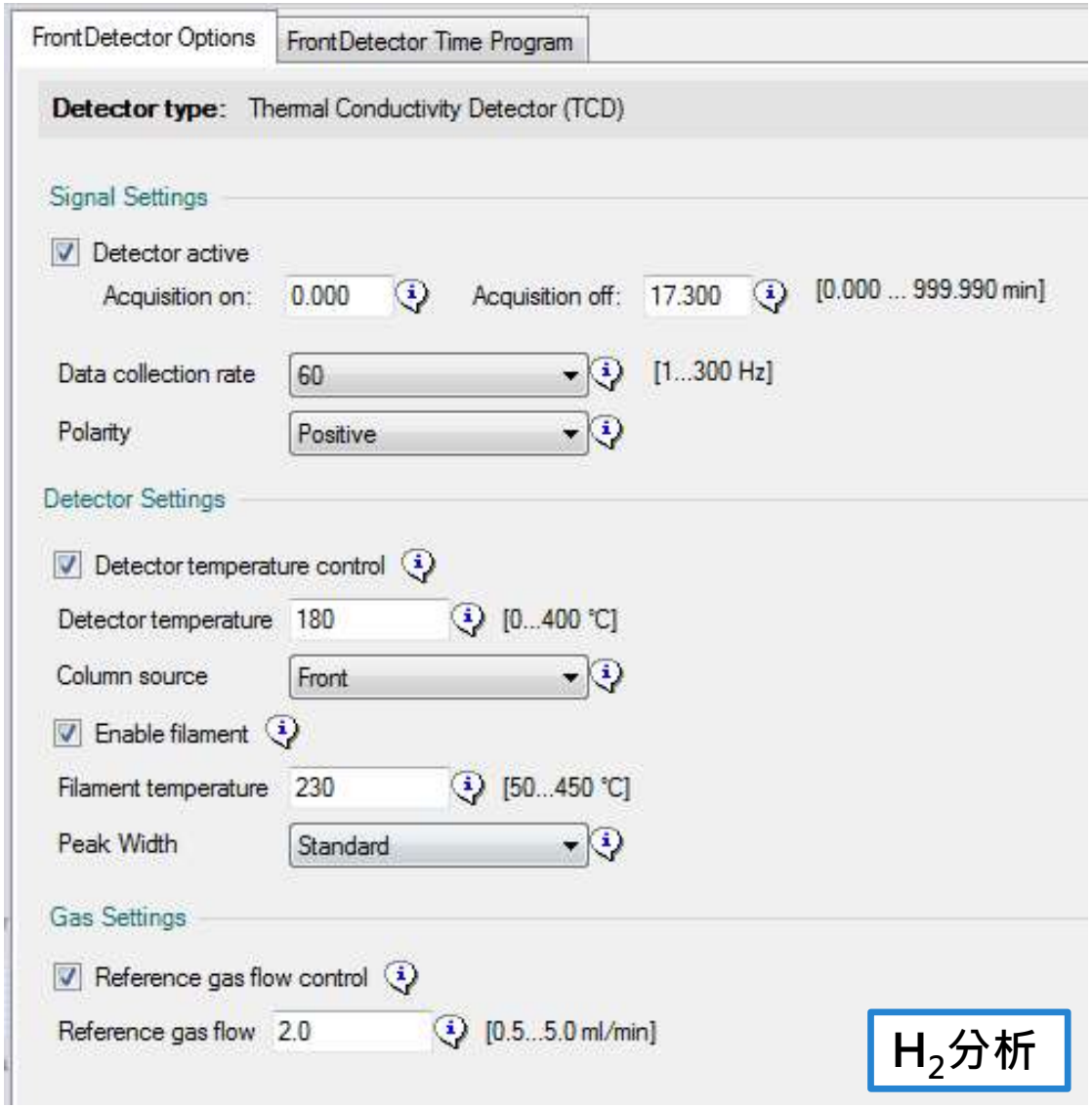
注意事項:

- 開啟Filament前，需確定有載流氣體通過 (5 – 10 min)
- 非必要時，不需打開Filament，以延長Filament壽命
- 避免分析過高濃度的鹵素化合物/ 酸性分析物
- 避免氧氣與空氣進入TCD，影響Filament壽命
- 更動TCD或換Column時，先把Filament關閉
- 訊號穩定就好 (因有Autozero)

Bake-out:

- 確認有載流氣體通過
- Filament溫度與TCD溫度升至300 °C
- 保持24 hr
- 如果無改善，請聯繫硬體工程師

TCD分析參數



- **TCD溫度: (越低越好)**
  - Filament T > Detector T
  - Delta T ≥ 50 °C (He) / ≥ 100 °C (N2)
  - Recommended: 200 °C
  - Lowest T: 150 °C

- **Acquisition time**
- **Data collection rate**
  - 30, 60 or 120 Hz
- **Polarity**
- **Reference gas flow:**
  - need to be optimized
  - 2 or 3 mL/min

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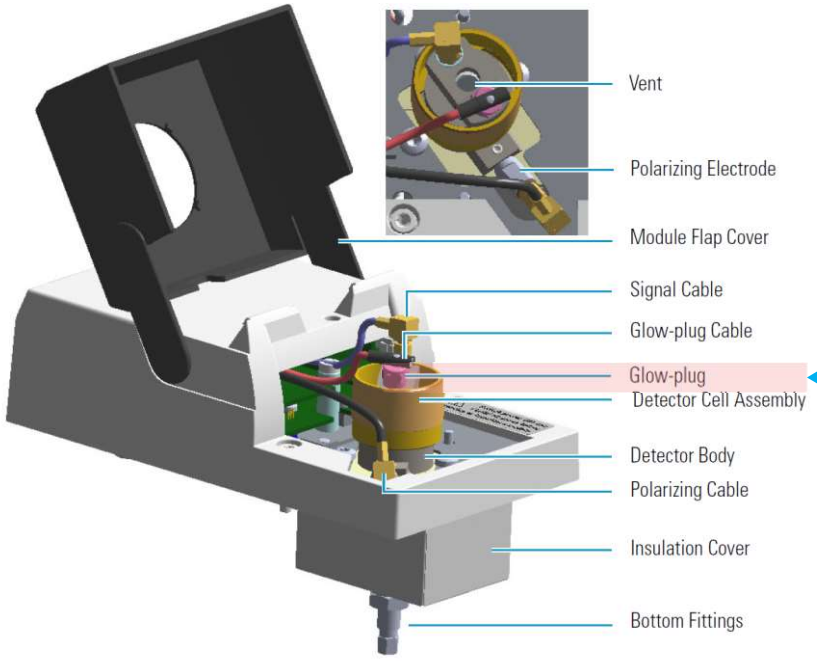
偵測器的保養與確認

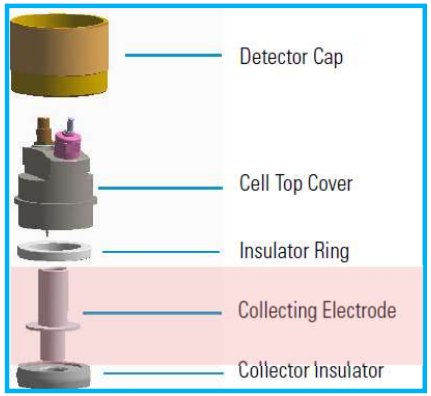
FID

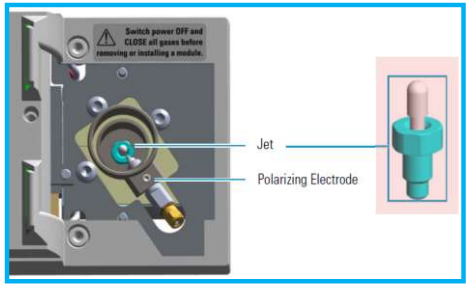
- **保持FID乾淨、無灰塵與沉積物**
- **訊號降低或雜訊上升**

定期保養:

- 清潔Collecting electrode (雜訊上升)
  - **清潔劑於超音波震盪5 min**
  - **用水與甲醇潤洗**
  - **於乾淨紙上，等待乾燥**
- 清潔FID Jet (特別是很髒的樣品)
  - **清潔方式同”清潔Collecting electrode”**
- 更換FID ignition glow-plug (若有必要時)







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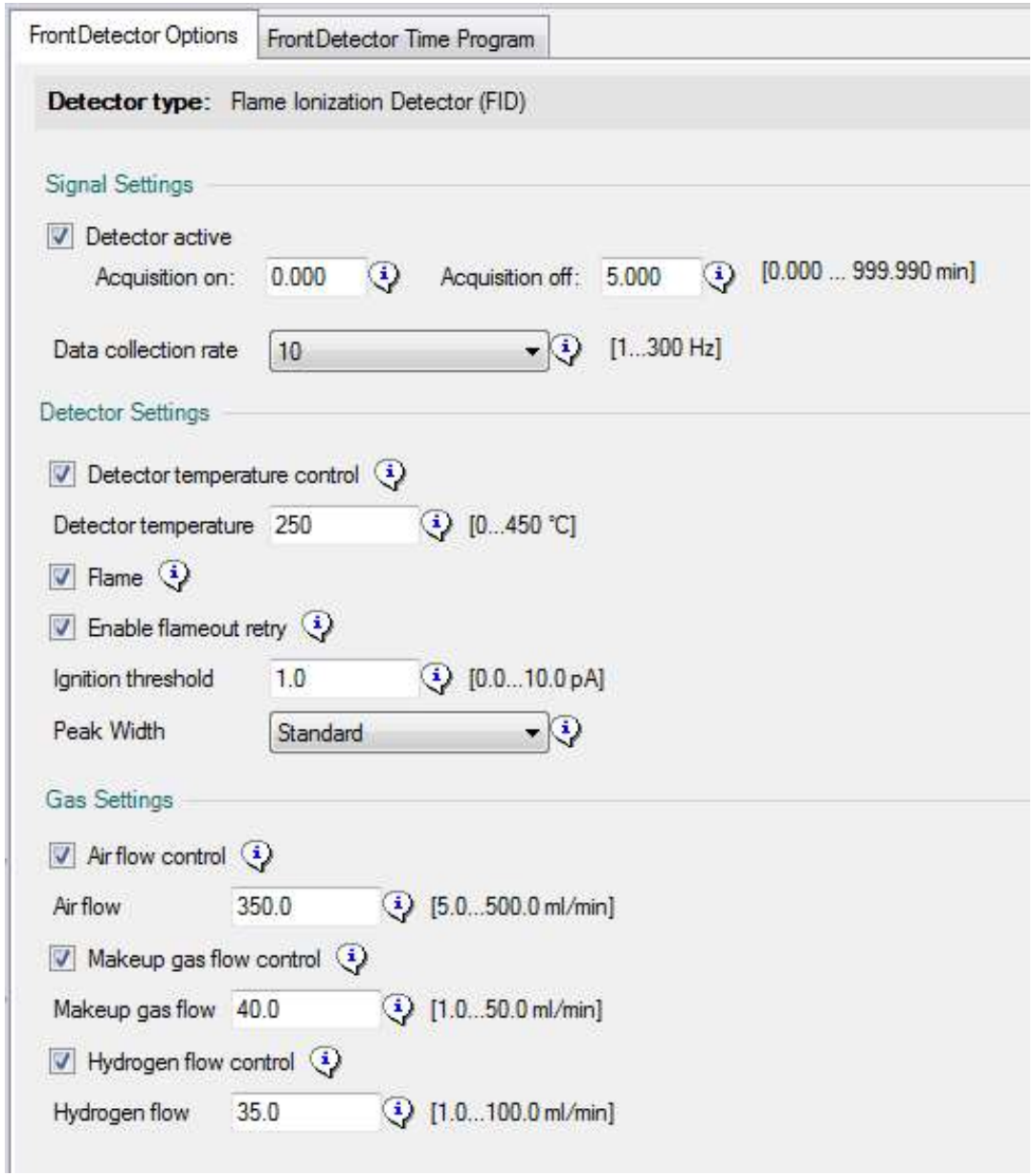
FID分析參數



- **FID點火時:**

  - Air Flow = 200 mL/min

  - H_2 Flow = 40 mL/min

- **Acquisition time**
- **Data collection rate**
- **Gas Settings**

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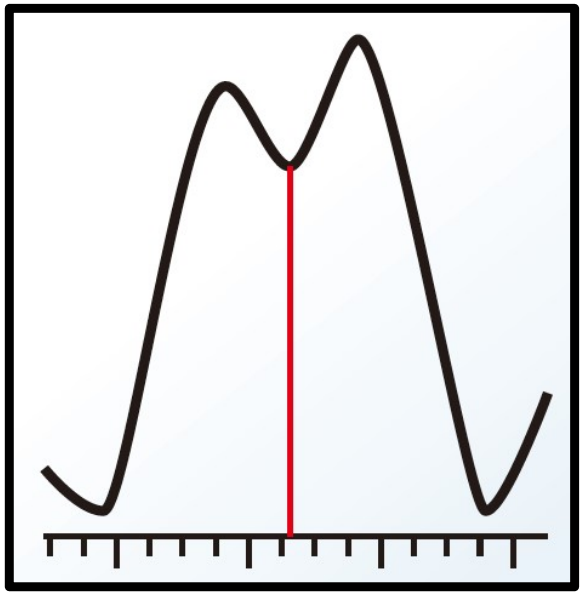
於偵測器端安裝管柱

不同偵測器的管柱安裝方式與深度

FID	NPD	TCD	ECD	FPD	PDD
Insert the column as far as goes and withdrawn about 2 -3 mm			23 mm	125 mm	136 mm

FAQ: Poor Resolution

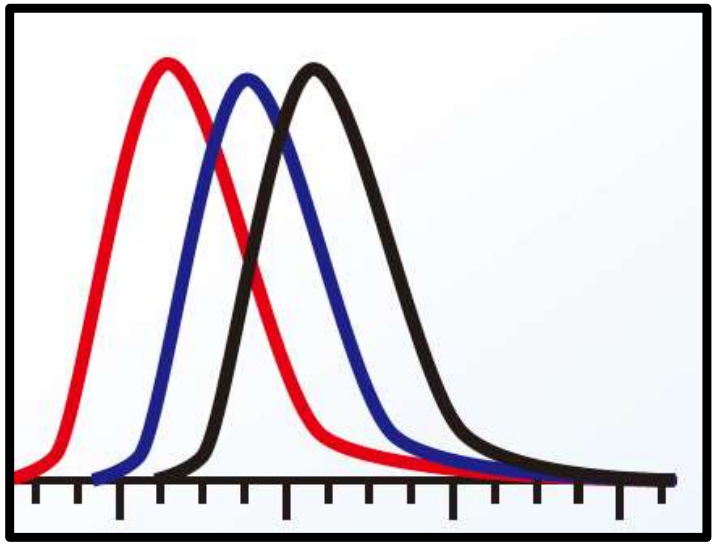


- **Non-selective stationary phase**
- **Poor efficiency**
- **Sample overload**
- **Incorrect analytical conditions used**

Solution:

- ✓ Choose appropriate column
- ✓ Adjust sample concentration or amount on column
- ✓ Verify temperature program, flow rates, and column parameters

FAQ: Poor RT reproducibility

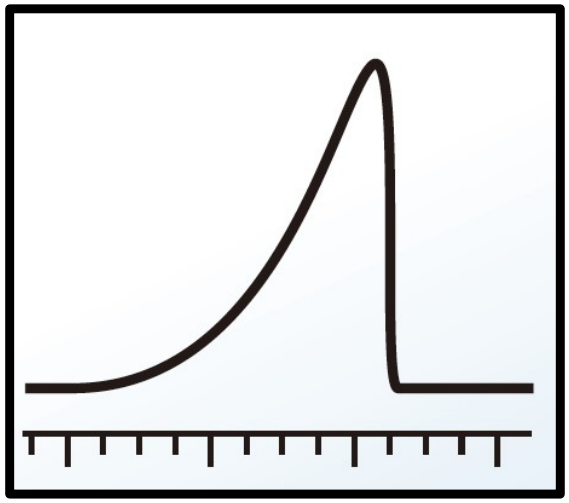


- **Leaks**
- **Analyte adsorption**
- **Incorrect column/oven program**
- **Manual injection time delay**
- **Incorrect or variable flow rate**
- **Incorrect oven equilibration time**
- **Column evaluation**

Solution:

- ✓ Leak check, replace septum
- ✓ Use properly deactivated liners, seals, and columns
- ✓ Verify temperature program
- ✓ Use autosampler or standardize manual injection precedure.

FAQ: Fronting peak

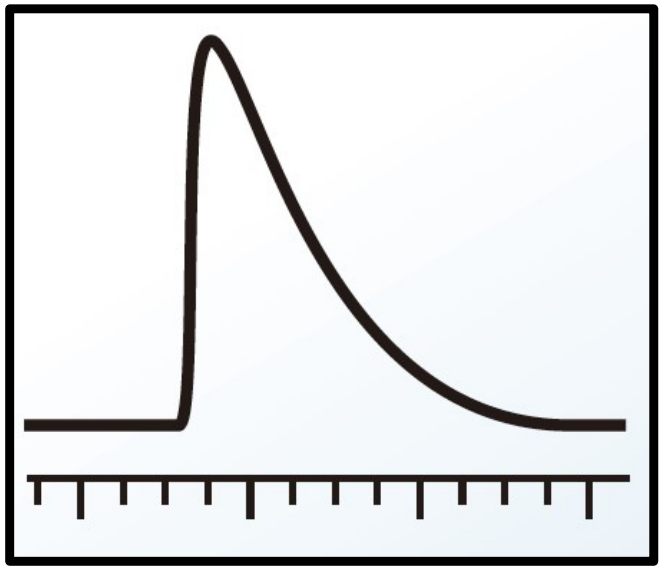


- **Incompatible stationary phase**
- **Column overloaded**

Solution:

- ✓ Choose appropriate column
- ✓ Reduce amount injected, dilute sample.
- ✓ Increase column inner diameter and/or film thickness.

FAQ: Tailing peak

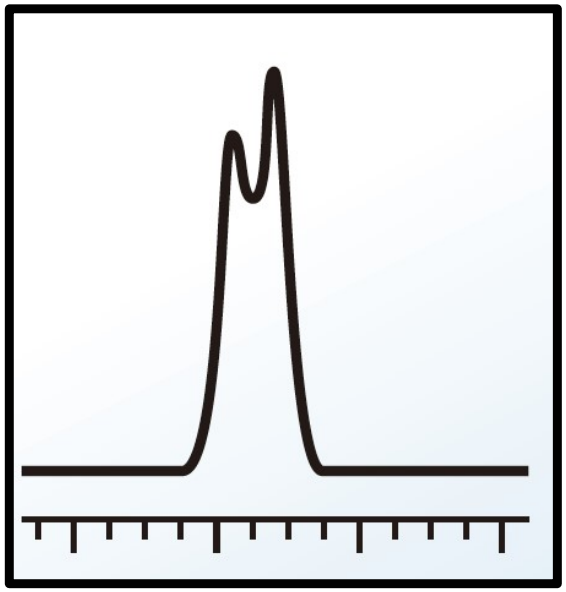


- **Adsorption due to surface activity or contamination**
- **Adsorption due to chemical composition of compound**
- **Needle hitting and breaking packing in inlet liner.**
- **Leak in system**
- **Inlet flow too low.**

Solution:

- ✓ Use properly cleaned and deactivated liner, seal, and column.
- ✓ Trim inlet end of column.
- ✓ **Derivatize compound.**
- ✓ Check for leaks at all connections, replace critical seals if needed.
- ✓ Check the flow rate

FAQ: Split peak

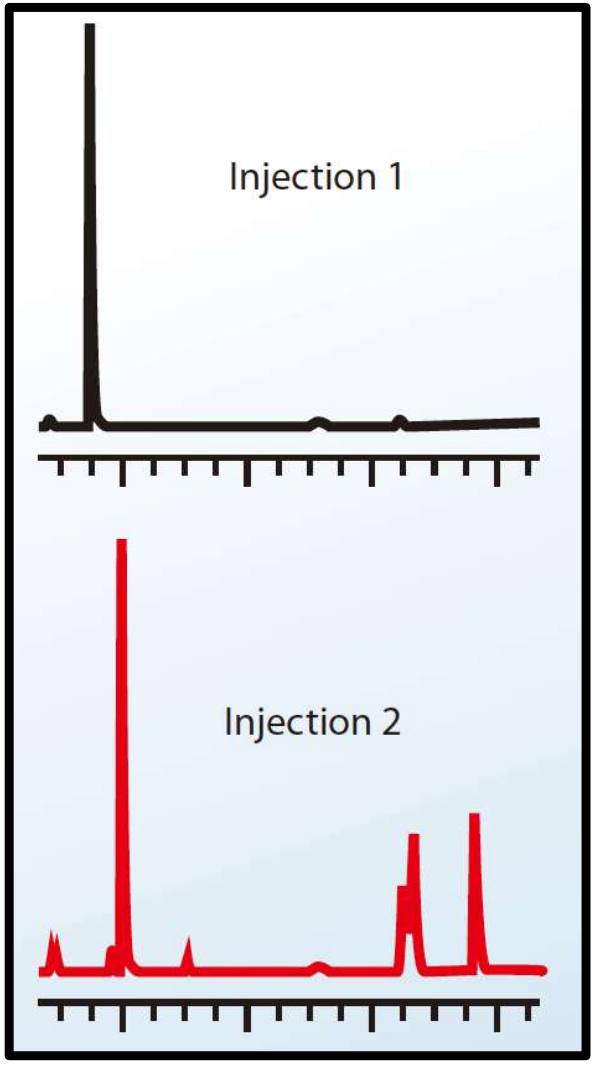


- **Mismatched solvent/stationary phase polarity**
- **Incomplete vaporization**
- **Sample loading capacity exceeded**
- **Fast autosampler injection into open liner**

Solution:

- ✓ Adjust solvent or stationary phase to allow wetting
- ✓ Add surface area, such as wool, to the inlet liner to enhance vaporization
- ✓ Use proper injector temperature
- ✓ Inject less sample
- ✓ Slow injection or use wool

FAQ: Carryover and Ghost peaks

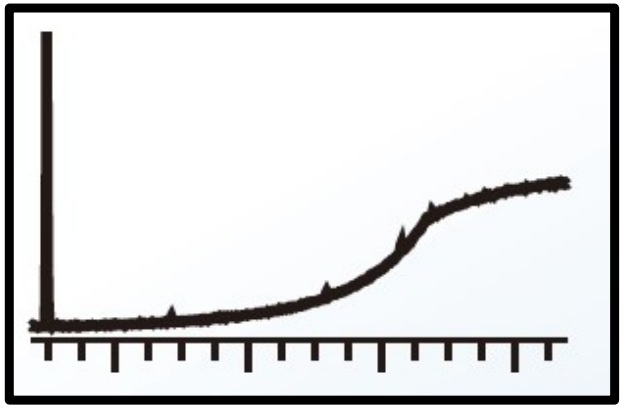


- **Contaminated syringe, rinse solvent, septum**
- **Sample volume exceeds liner volume**
- **Last analysis ended too soon**

Solution:

- ✓ Replace rinse solvent, syringe, septum
- ✓ Use a liner with a large internal diameter
- ✓ Increase split flow.
- ✓ Use slower injection rate
- ✓ Use pressure-pulse injection
- ✓ Extend analysis time to allow all components and/or matrix interferences to elute
- ✓ Column condition

FAQ: Column bleed

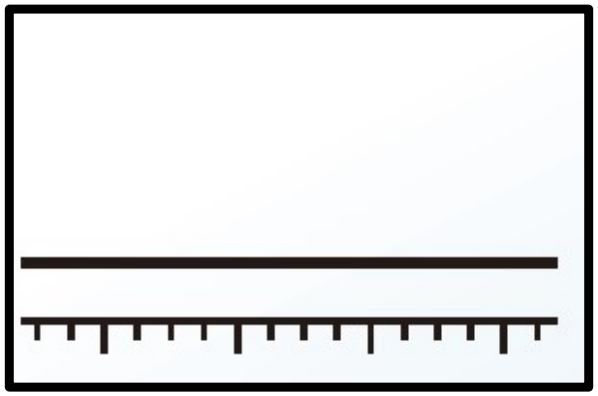


- Improper column conditioning
- Contamination
- Leak in system and oxidation of stationary phase
- Polar stationary phase
- Film thickness

Solution:

- ✓ Increase conditioning time and/or temperature.
- ✓ Trim column and/or heat to maximum temperature to remove contaminants.
- ✓ Replace carrier gas and/or detector gas filters.
- ✓ Clean injector and detector
- ✓ Use slower injection rate
- ✓ Check for oxygen leaks across the entire system and replace seals and/or filters.
- ✓ Replace column

FAQ: No peaks

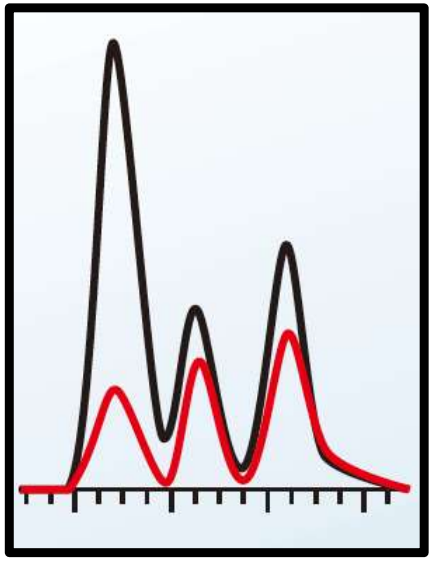


- **Injection problems**
- **Broken column**
- **Column installed into wrong injector or detector**
- **Detector problems**

Solution:

- ✓ Plugged syringe; clean or replace syringe.
- ✓ Verify there is sample in the syringe.
- ✓ Injecting into wrong inlet
- ✓ Verify carrier gas is flowing
- ✓ Replace column or re-install column
- ✓ Signal not recorded; check detector cables and verify that detector is turned on.
- ✓ Detector gas turned off or wrong flow rates used; turn detector on and/or adjust flow rates.

FAQ: Response variation



- **Sample issues**
- **Syringe problems**
- **Electronics**
- **Dirty or damaged detector**
- **Flow/temperature settings wrong or variable**
- **Leaks**

Solution:

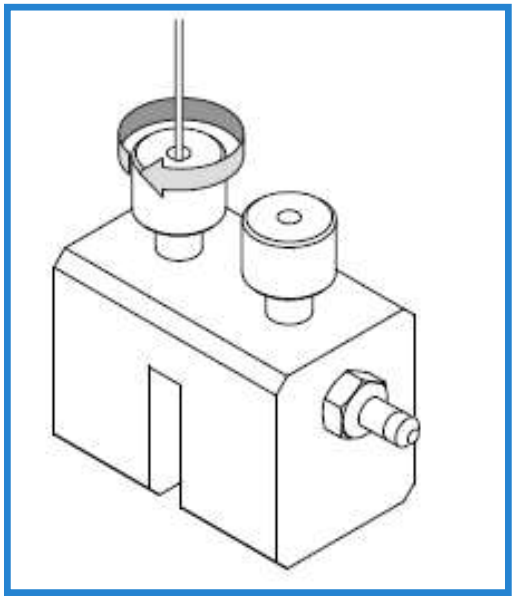
- ✓ Check sample
- ✓ Leak check
- ✓ Replace syringe or check autosampler operation
- ✓ Perform detector maintenance or replace parts
- ✓ Replace deactivated liner
- ✓ Check that split ratio is correct

如何更換GC管柱 – 更換管柱步驟 (用手鎖緊後，以板手轉1/4圈)

1. 將原本的管柱，以合適的板手將**注射口 Nut** 與**偵測器Nut** 轉鬆後，卸下管柱
2. 先連接新管柱與注射口端 (**SSL: 5mm for SLess & 10mm for Split ; PTV: 30 mm**)
3. 開啟 GC 流速，並將管柱尾端放置於乾淨有機溶劑內，確認有氣泡跑出
4. 於 Column Properties 的位置，設定新管柱的規格

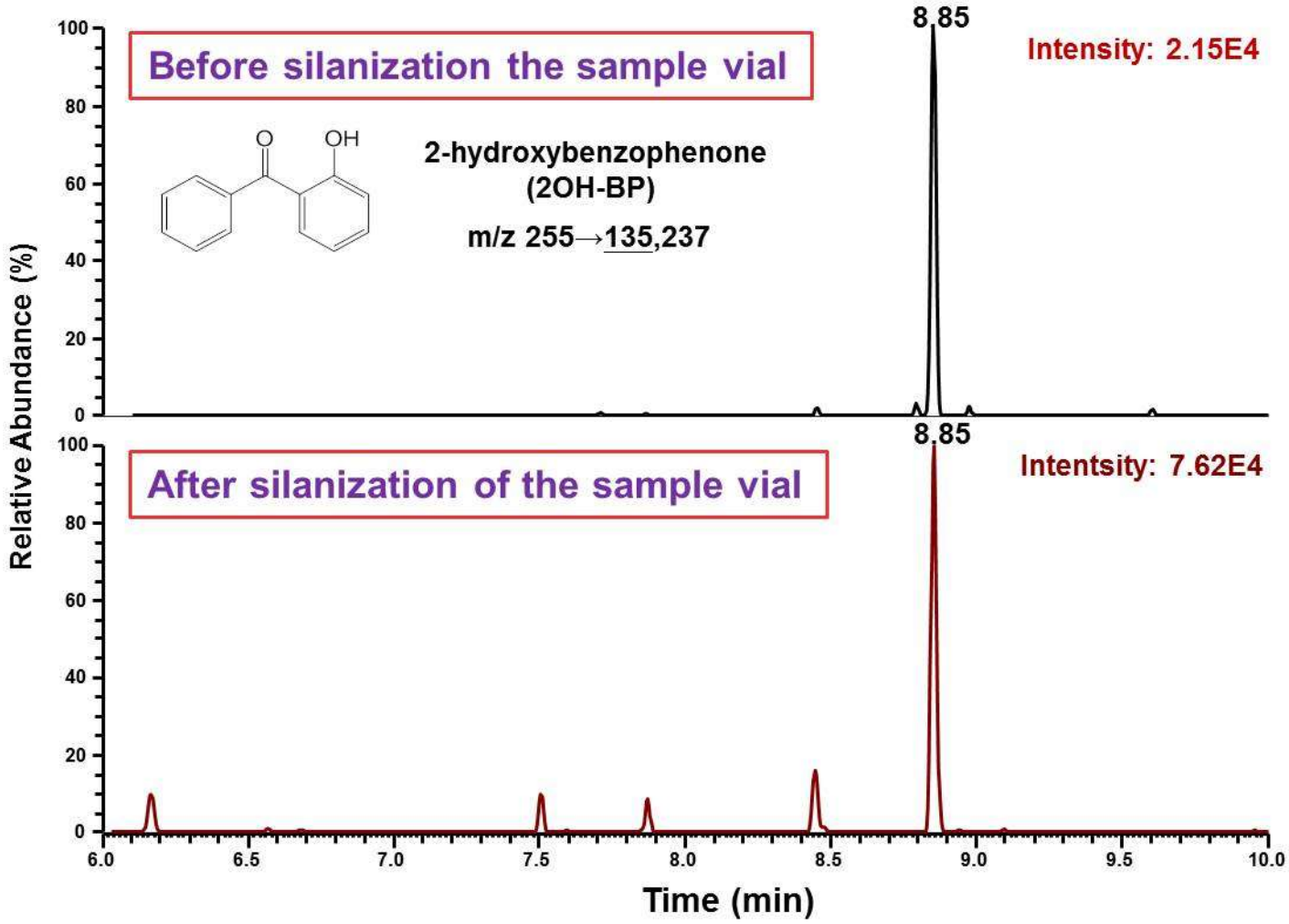
   - Chromeleon: *Chromeleon Console > Instruments > Front Inlet/ Back Inlet*

5. 進行 Leak Check，通過後再安裝管柱至偵測器端



小技巧

- 超微量分析: 樣品瓶的去活化
- 有機金屬分析: 樣品瓶酸洗 (Acidic wash)



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