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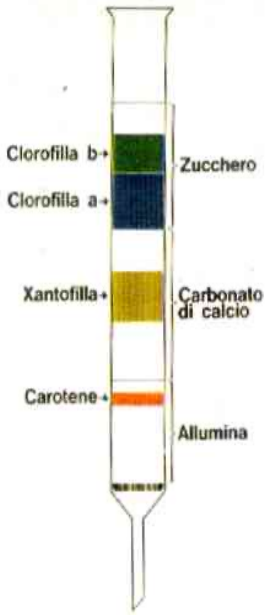
Iniettori e Liners, best pratic

dott. Davide Facciabene
GC & GC-MS Product Specialist at Thermo Fisher Scientific

Bari – 17 Novembre 2017

STORIA DELLA CROMATOGRAFIA

Il nome più importante e noto al quale associare il concetto di cromatografia è quello del botanico russo Mikhail Tswett (1872-1919). Nel **1906** pubblicò un libro dove descriveva la separazione e l'isolamento dei pigmenti clorofilliani presenti nei cloroplasti delle cellule algali.



Egli usò questa tecnica per separare i differenti pigmenti algali facendoli passare attraverso una colonna di vetro impaccata con particelle fini di carbonato di calcio: le specie venivano separate dalla pressione esercitata dal solvente (etere di petrolio). Mentre il solvente passava attraverso la colonna i pigmenti si muovevano e si separavano apparendo come bande colorate con diverse gradazioni dal giallo al verde. Fu per questo motivo che chiamò il processo "**cromatografia**": deriva dall'unione di 2 parole greche chroma che significa "colore" e graphein che significa "scrivere"

Tsweett è il responsabile della maggior parte della nomenclatura che attualmente viene utilizzata in cromatografia.

NOBEL & CROMATOGRAFIA

Le sue applicazioni sono cresciute in modo vertiginoso nell'ultimo mezzo secolo, non solo a causa dello sviluppo di nuove tecniche cromatografiche ma anche dal crescente bisogno da parte degli scienziati di metodi sempre più efficaci per la caratterizzazione di miscele complesse. L'impatto incredibile che queste tecniche hanno avuto per la scienza è testimoniato dall'assegnazione del premio Nobel nel **1952** a J.P. Martin e R.M. Synge per le loro scoperte in questo campo. E dalla serie di dodici premi Nobel assegnati tra il **1937** e il **1972** per lavori in cui la cromatografia riveste un ruolo fondamentale.

DEFINIZIONE DI CROMATOGRAFIA

Proposta dall'International Union of Pure and Applied Chemistry (IUPAC) nel 1993:

"la cromatografia è un metodo fisico di separazione nel quale i componenti da separare vengono distribuiti tra due fasi: una denominata stazionaria e l'altra mobile."

... è il mezzo più diffuso per realizzare separazioni analitiche di componenti chimici presenti in una miscela...

La cromatografia è un metodo molto efficace e trova applicazioni in tutte le branche della scienza. Può separare gas, sostanze volatili attraverso la GasCromatografia (GC), i composti non volatili e i materiali polimerici incluse le sostanze biologiche attraverso la Cromatografia Liquida (LC)

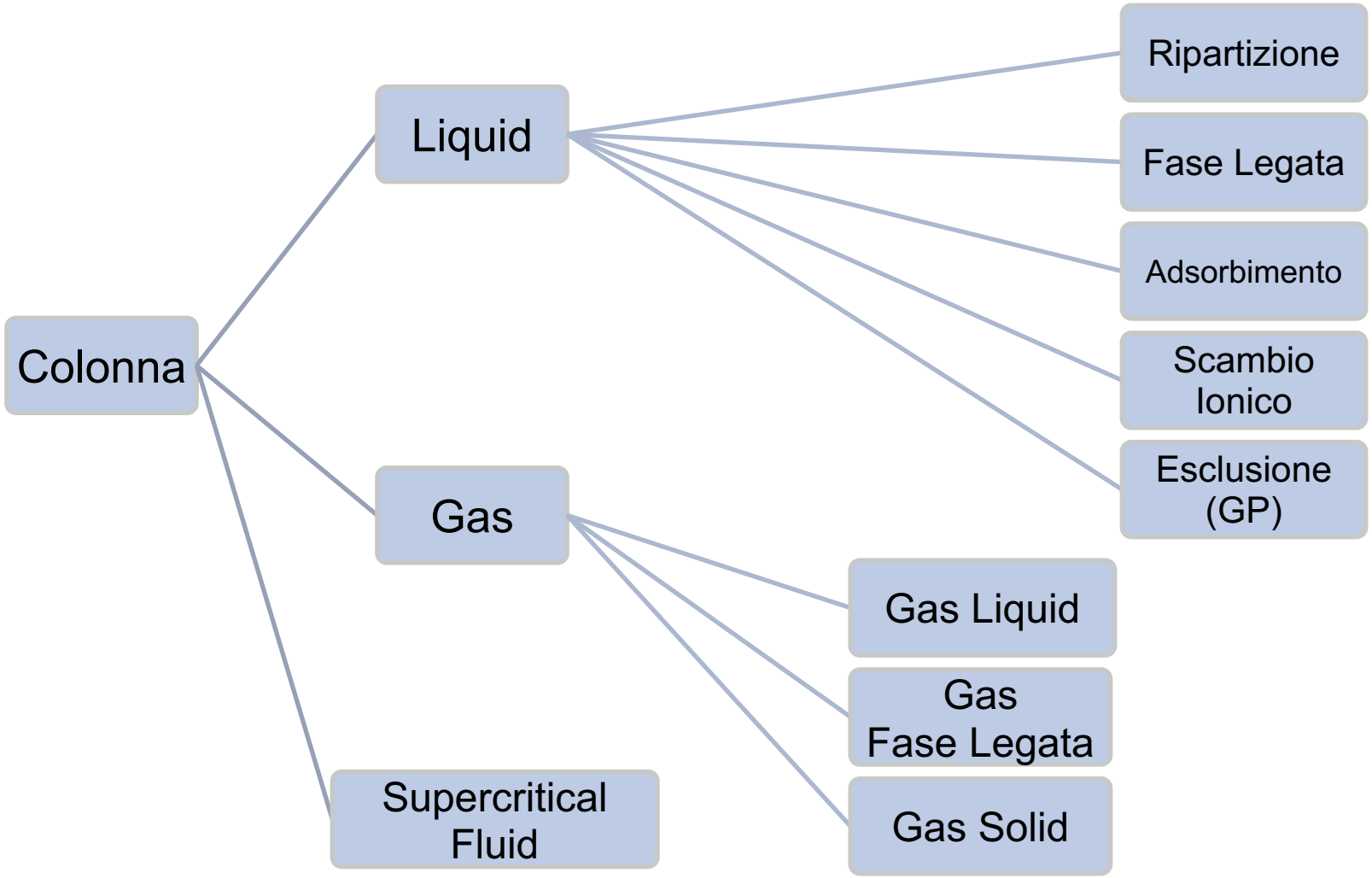
CROMATOGRAFIA

In tutte le separazioni cromatografiche il campione viene disciolto in una **fase mobile**, che può essere un gas, un liquido o un fluido supercritico. Questa fase mobile viene poi fatta passare attraverso una **fase stazionaria**, immiscibile, riposta in una colonna o su una superficie solida.

Ogni singolo analita contenuto nel campione in esame, presenta una diversa affinità sia con la fase mobile che con la fase stazionaria. Questa differenza permette la separazione dei singoli composti. Tecnicamente parlando si definisce “RISOLUZIONE” di una miscela.

Alla base della cromatografia ci sono tutta una serie di principi fisici che ne classificano le varie tecniche (GLC, GSC, Ripartizione, Adsorbimento, ecc. ecc.).

CROMATOGRAFIA



GASCROMATOGRAFO

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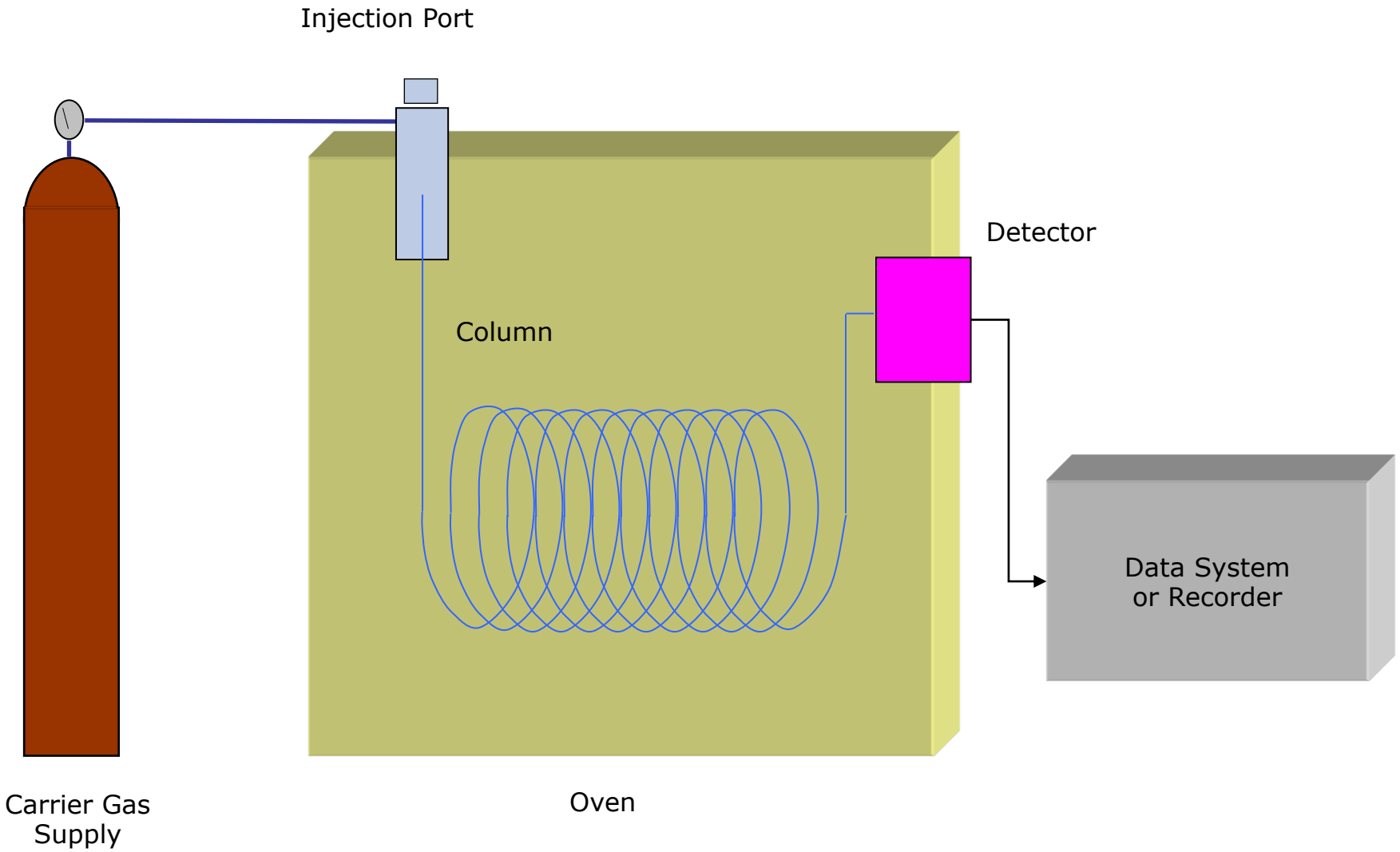
SCHEMA DI UN GASCROMATOGRAFO



GASCROMATOGRAFIA

UN COMPOSTO PER POTER ESSERE ANALIZZATO IN GC

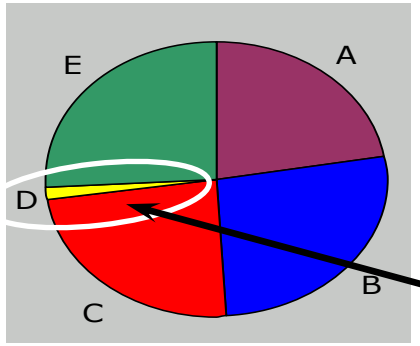
DEVE AVERE SUFFICIENTE VOLATILITA' E STABILITA' TERMICA

Ciò significa che devono avere un'apprezzabile volatilità a temperature uguali o inferiori a 300-400 °C e, a queste temperature, devono vaporizzare senza decomporsi e/o reagire.

La volatilità di un composto dipende, oltre che dal punto di ebollizione, dal peso molecolare ma anche dalla polarità.

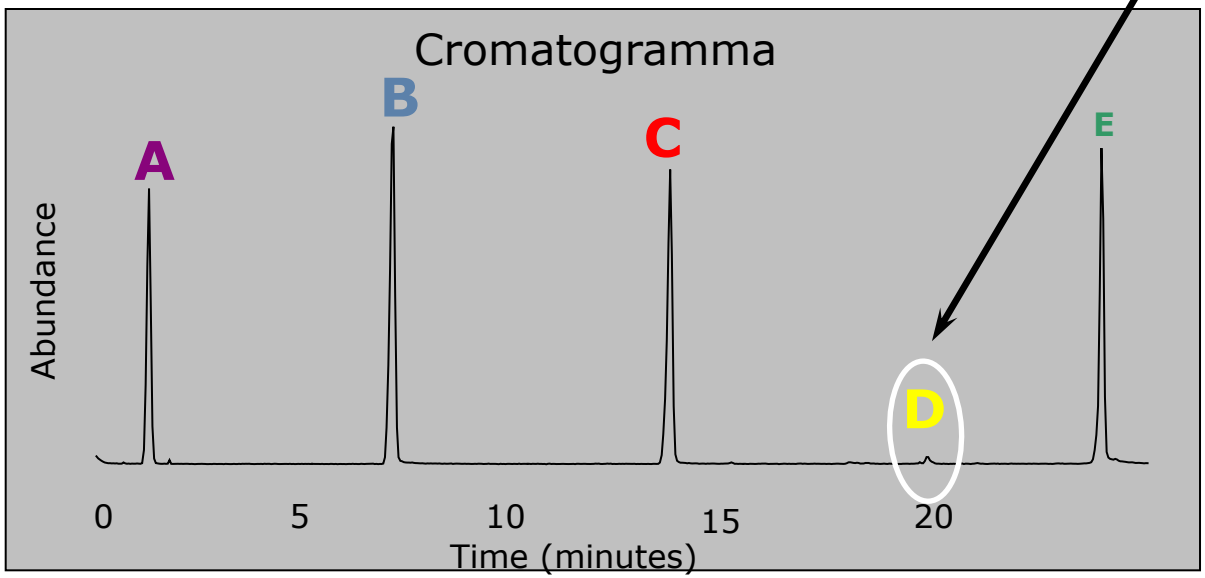
GASCROMATOGRAFIA

GC



Campione

L'abbondanza di un picco (area o altezza) è proporzionale alla concentrazione della sostanza nel campione in esame.



Il tempo impiegato da una sostanza per uscire dalla colonna è definito come TEMPO di RITENZIONE, ed è un dato caratteristico.

PROGRAMMA TERMICO

Fa sì che tutti gli analiti vengano eluiti in un tempo ragionevole, ottenendo:

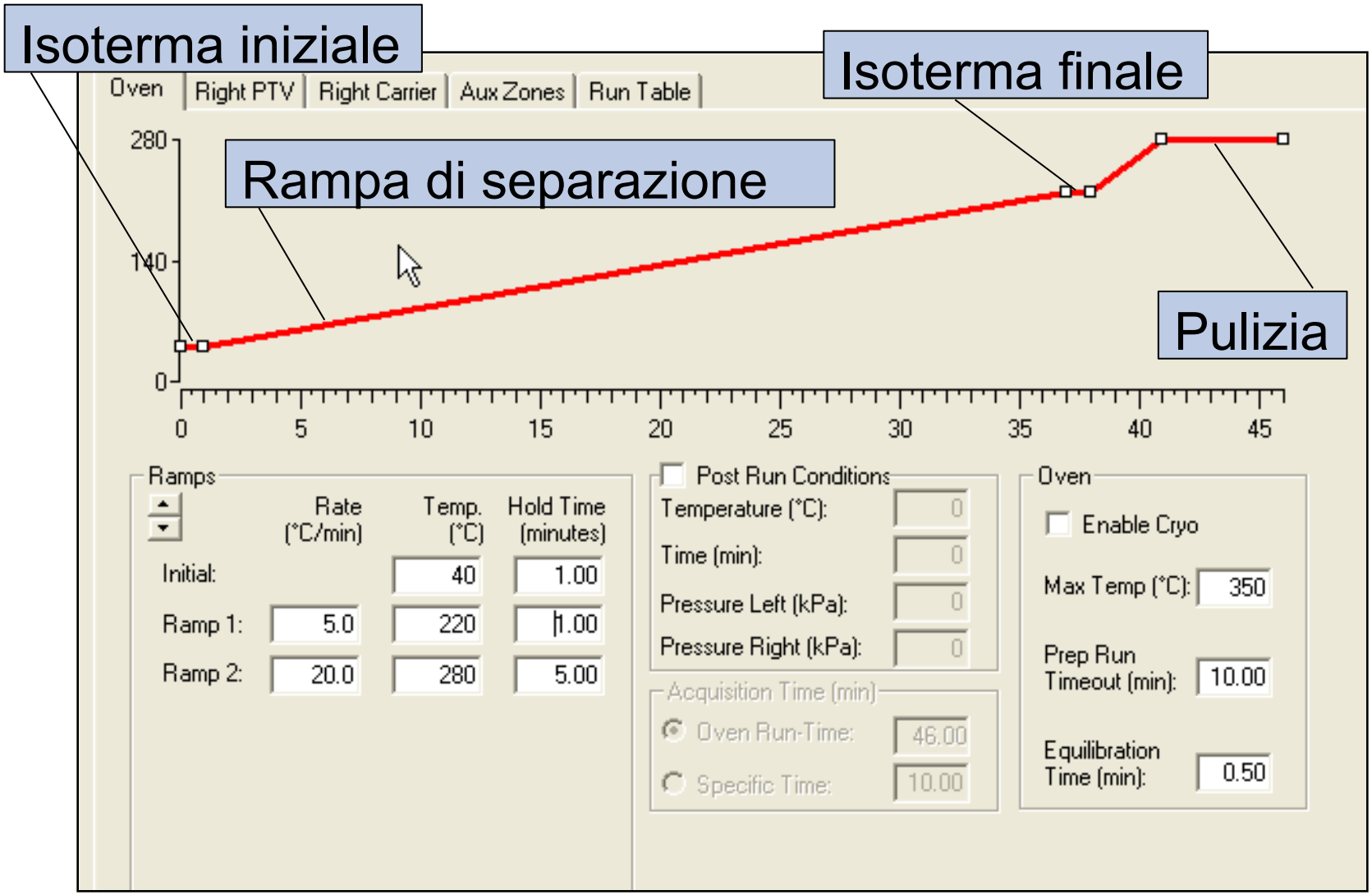
☞ Tempi brevi, anche quando l'intervallo di punti di ebollizione degli analiti è molto ampio (sia basso bollenti che alto bollenti)

☞ Picchi di larghezza simile per tutti gli analiti

☞ Picchi di altezza comparabile per tutti gli analiti (discriminazione minimizzata)

☞ Picchi simmetrici

PROGRAMMA TERMICO



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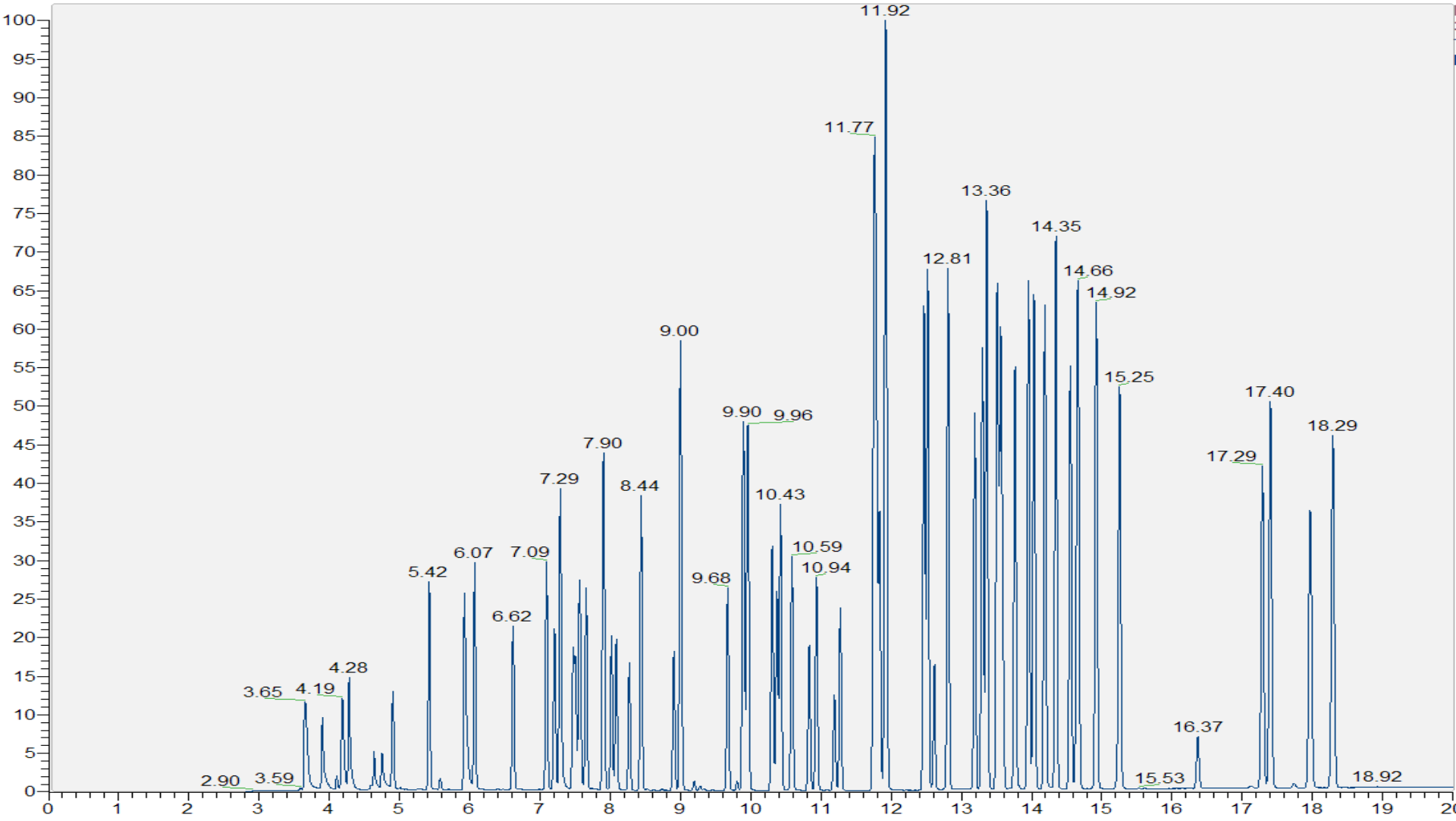
GASCROMATOGRAFIA



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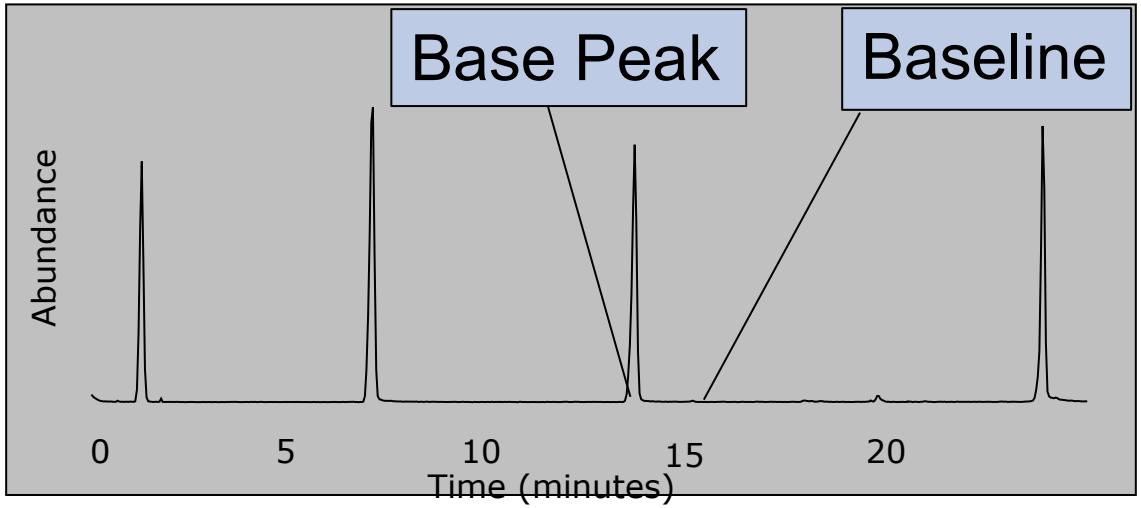
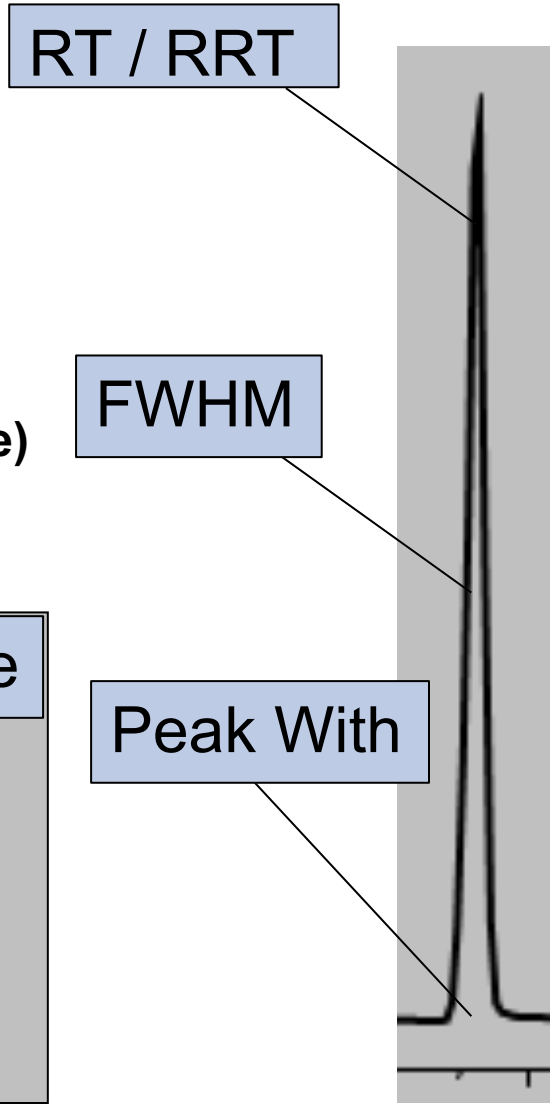
NOMENCLATURA

Baseline : Linea di Base
Peak : Picco (area/altezza)
Base Peak : Base del picco
Peak With : Larghezza alla base del picco (sec)
FWHM : Larghezza a metà altezza del picco (sec)
S/N : Rapporto segnale/rumore (RMS)
RT : Tempo di ritenzione (min, centesimi di sec)
RRT : Tempo di ritenzione relativo (adimensionale)
RF : Response Factor (area / concentrazione)
RRF : Relative RF (adimensionale)

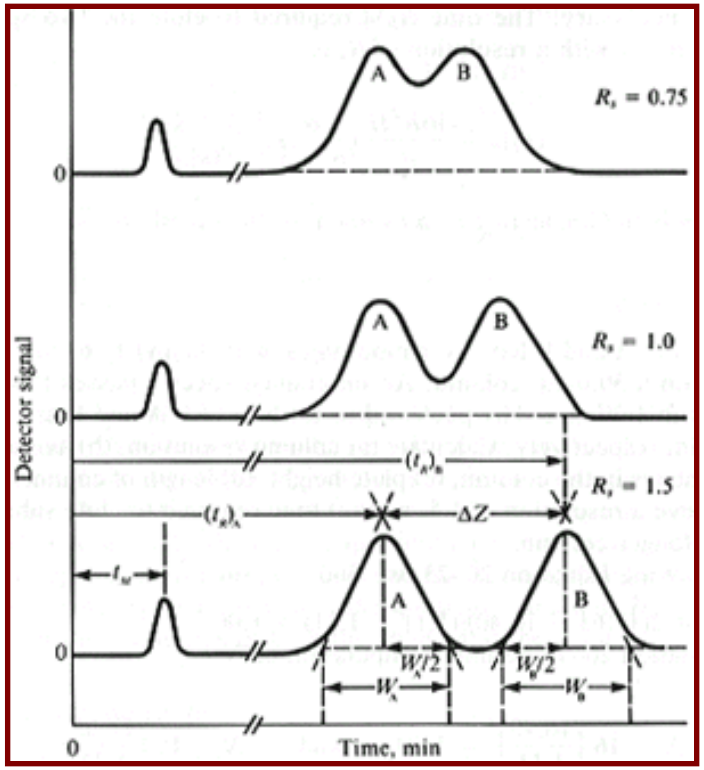




RISOLUZIONE

La risoluzione di una colonna (R) è la misura quantitativa della sua efficienza nella separazione di due analiti

$$R = \frac{2\,(t_{R2} - t_{R1})}{(w_{b1} + w_{b2})}$$



R = 1 i due picchi aventi aree uguali sono separati approssimativamente al 98% della base

R = 1,5 i due picchi aventi area uguale sono separati al 99,7%.

Le colonne capillari generalmente presentano risoluzione migliore di quelle impaccate.

Un aumento o della temperatura o di pressione diminuisce la risoluzione

EFFICIENZA

È importante ricordare che i piatti teorici non esistono realmente; essi sono immaginari e servono solo per meglio far capire come lavora una colonna. Servono anche per ottenere una misura quantitativa dell'efficienza della colonna stessa, per far ciò si utilizzano due parametri tra loro correlati:

Altezza di un piatto teorico (H)

$$H = L/N$$

L è la lunghezza del riempimento della colonna (cm) .

N il numero di piatti teorici in una colonna.

Il numero di piatti teorici (N)

$$N = 5{,}54\ t_R^2/W_{h/2}^2$$

$W_{h/2}$ è la larghezza del picco presa a metà altezza

L'efficienza di una colonna aumenta all'aumentare dei piatti teorici, diminuisce cioè con l'altezza di un singolo piatto.

LUNGHEZZA DI UNA COLONNA

Efficienza media e tempi di ritenzione delle colonne GC in funzione della lunghezza

Lunghezza (m)	N	t_R (min)
30	155000	15.2
60	304000	36.8
120	550000	82.4
150	719000	125.0

Colonna lunghe aumento dell'efficienza ma anche aumento della durata delle analisi

DIAMETRO INTERNO DI UNA COLONNA

Efficienza tipica delle colonne GC in funzione del diametro interno

	ID (mm)	N
Colonne capillari	0.20	5000
	0.25	4200
	0.32	3300
	0.53	1600
	0.75	1200
Colonne impaccate	2	2000

se si riduce il diametro l'efficienza migliora.

MIGLIORAMENTO DELLA SEPARAZIONE

La Risoluzione (R) o separazione dei picchi cromatografici dipende da:

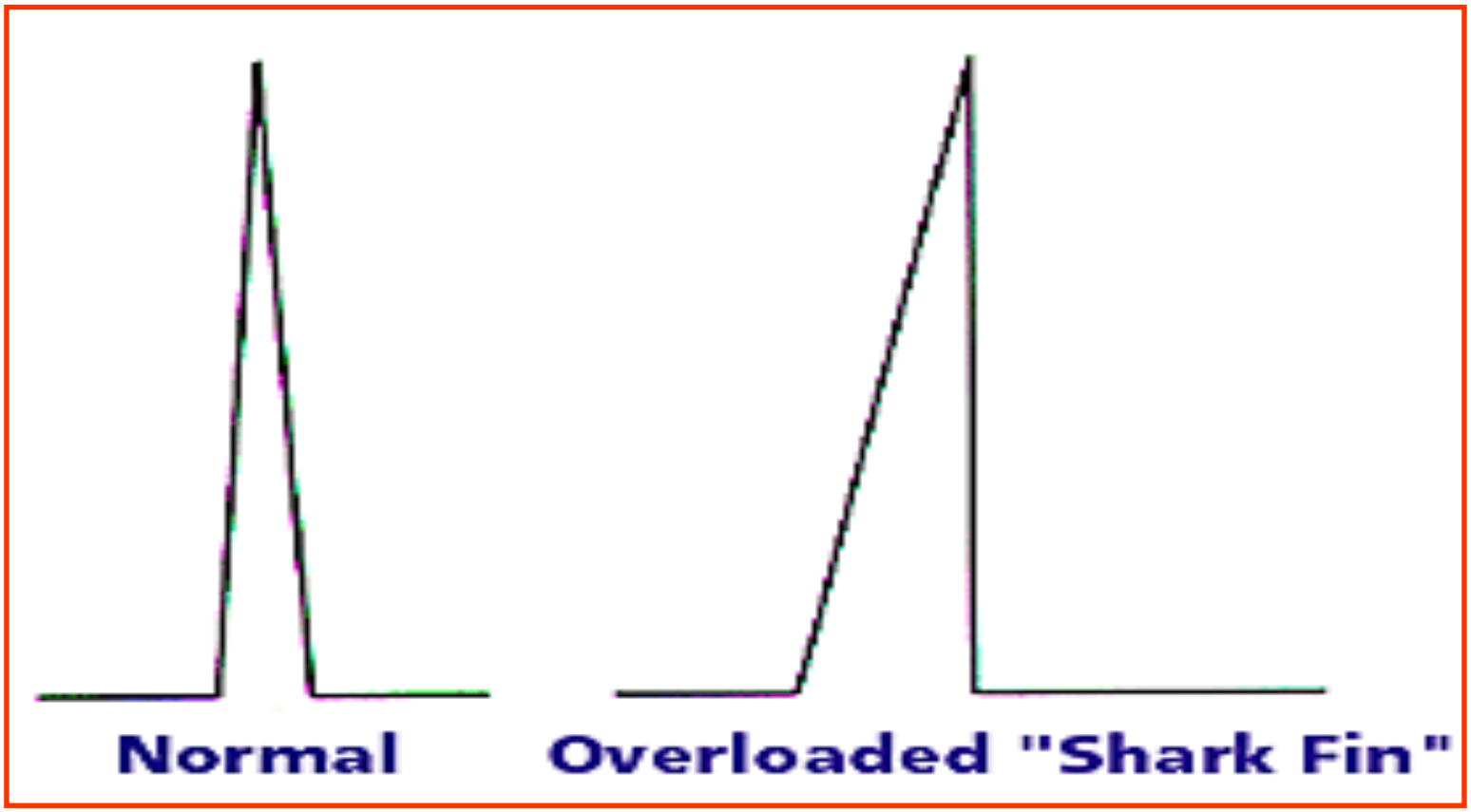
- **Scelta fase stazionaria**
- **Scelta fase mobile (***migliore H2, He, N2 peggiore***)**
- **Programma di temperatura**
- **Lunghezza colonna**

L' Efficienza (H) o allargamento dei picchi cromatografici dipende da:

- **Diametro colonna (***migliore con ID più piccolo***)**
- **Riempimento della colonna (***film tickness, particle size***)**
- **Velocità fase mobile (**migliora all'aumentare di v)

FORMA DEL PICCO CROMATOGRAFICO

NORMALE **SOVRACCARICO**



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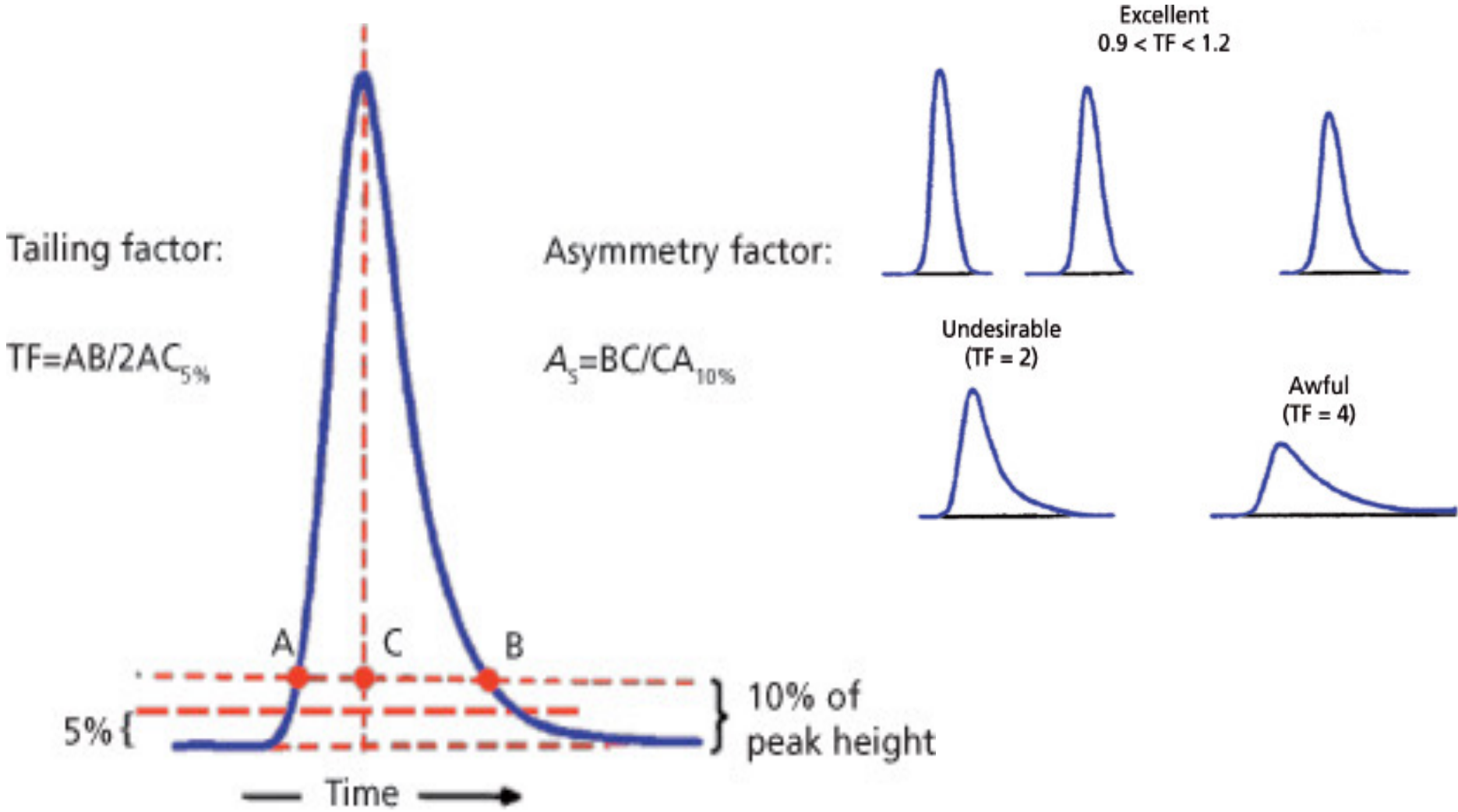
FATTORE del PICCO CODATO o di ASIMMETRIA



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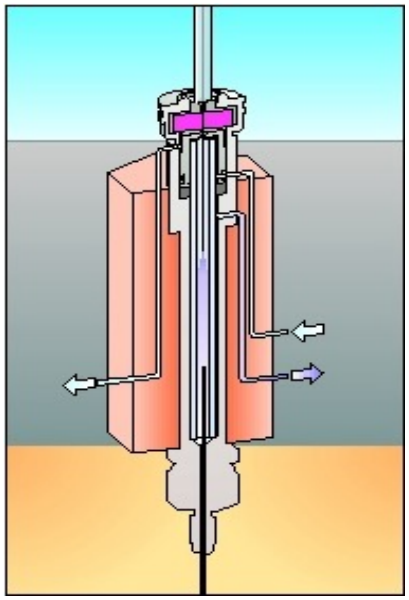
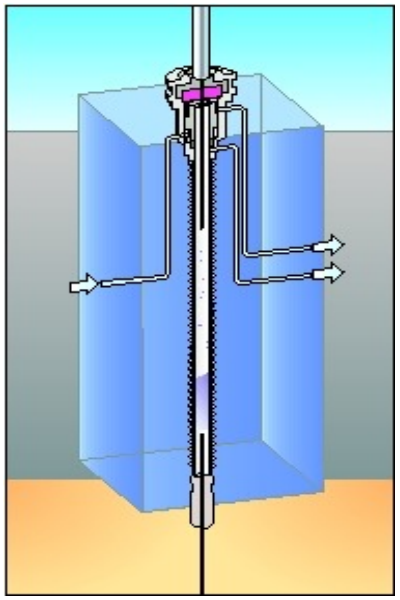
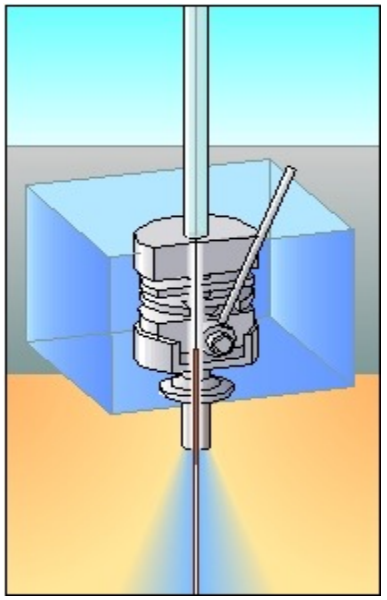
INIETTORI



SPLIT/SPLITLESS

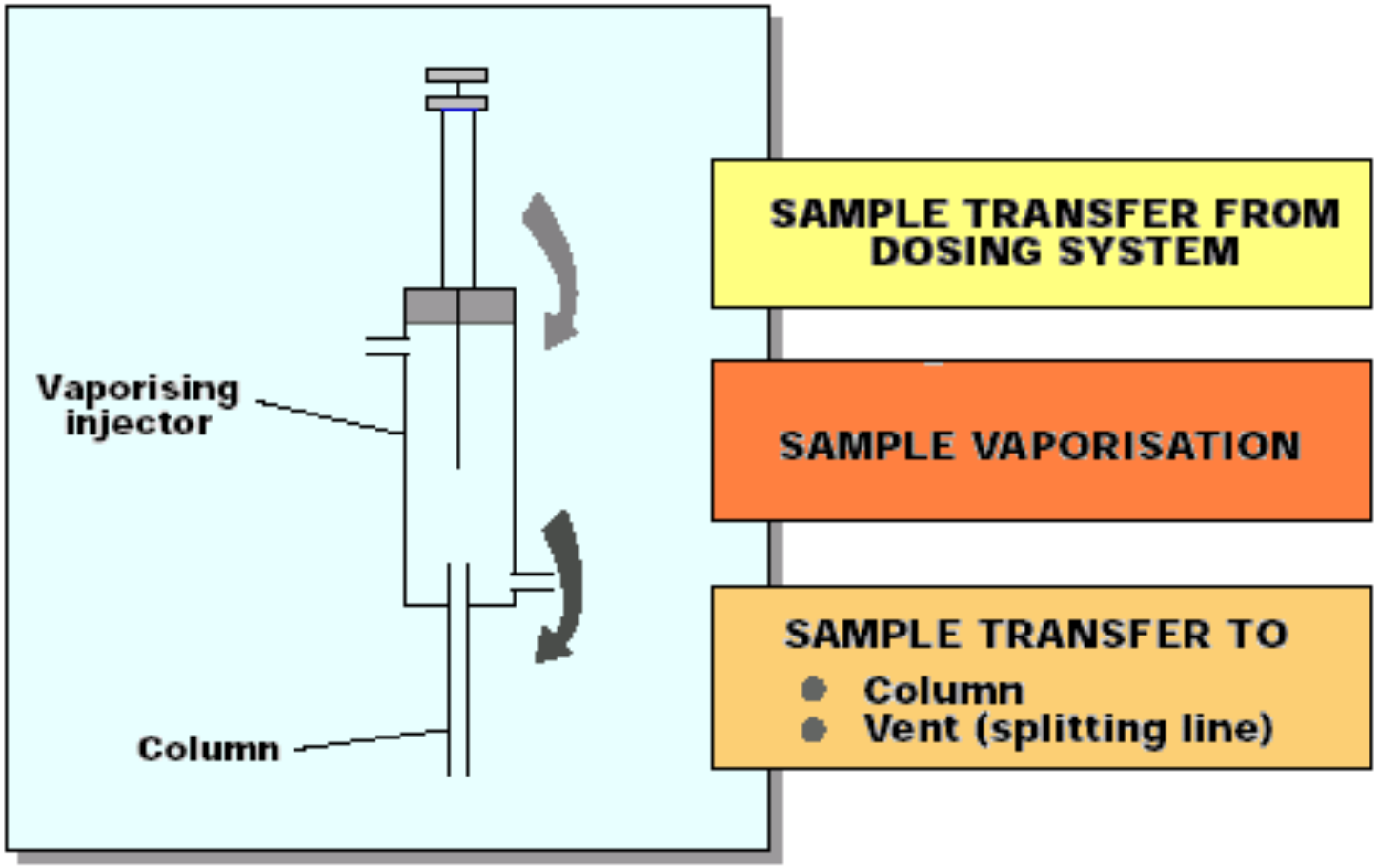


PTV



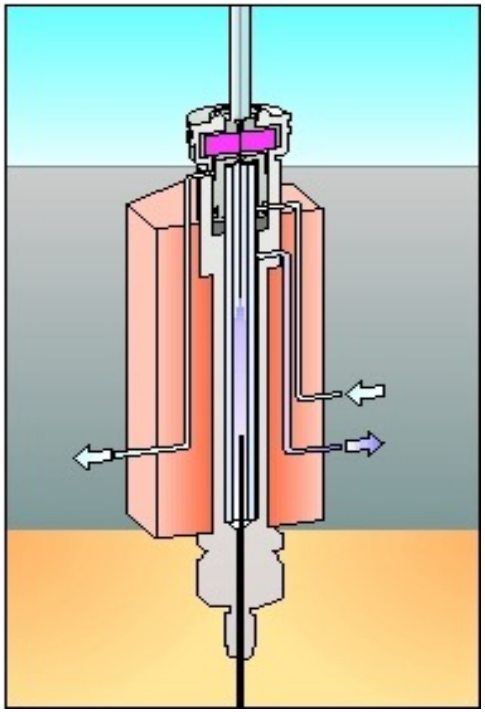
ON COLUMN

Vaporizing Injectors - Sample Transfer Process



SPLIT / SPLITLESS (SSL)

Iniettore a temperatura costante, particolarmente diffuso, relativamente semplice nell'utilizzo e nello sviluppo del metodo strumentale, ma poco versatile.



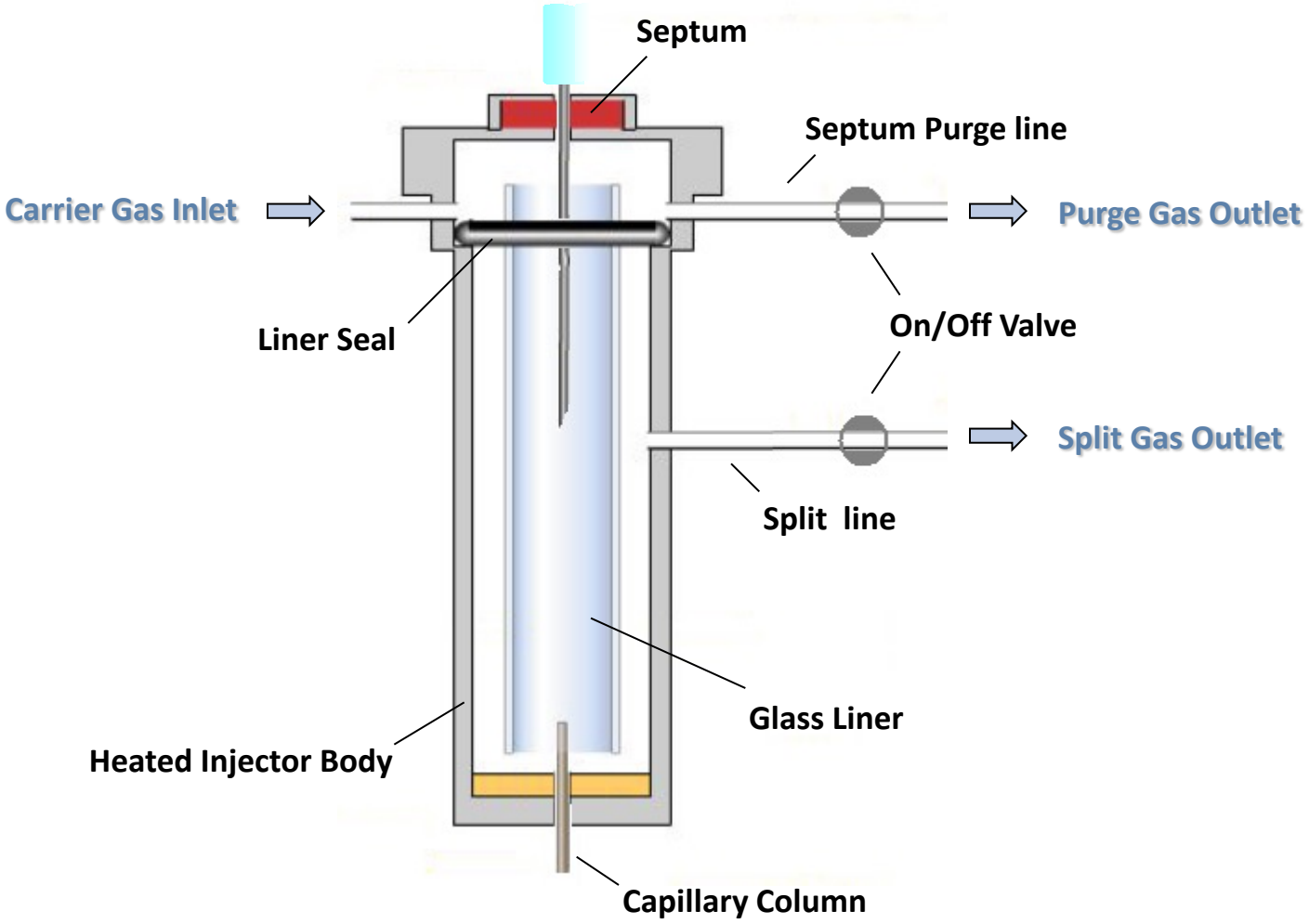
Modalità di iniezione split e splitless

Piccoli volumi di iniezione :

1 – 3 ul liquidi o 0,1 a 2,5 ml in spazio di testa

Temperatura di lavoro fino a 400°C

SSL Concept



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INIEZIONE SPLIT

Solo una frazione del campione viene iniettato in colonna.

L'SSL è costituito da una camera di iniezione termostatata a elevata temperatura, in cui il campione evapora rapidamente e si miscela con il gas di trasporto.

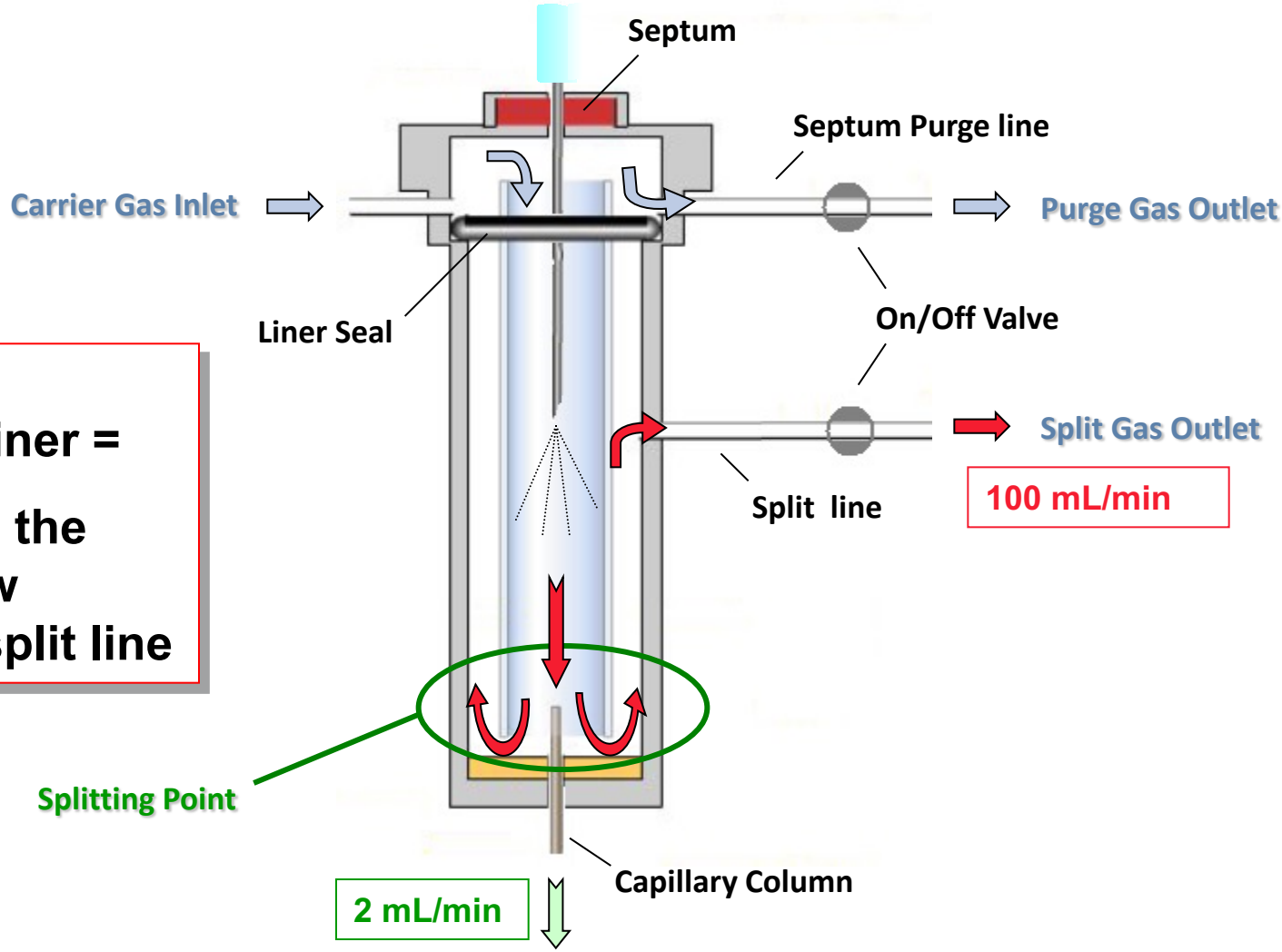
All'uscita la miscela di distribuisce tra l'ingresso in colonna e il condotto di split, secondo i rapporti impostati dall'operatore agendo su una valvola.

Il flusso del gas di trasporto viene suddiviso in due parti: una diretta verso il setto che chiude la camera di iniezione per mantenerlo sempre pulito (flusso di purge: 5-10 ml/min regolato dalla valvola di Purge); l'altra, invece, trascina il campione parte in colonna e parte verso il condotto esterno (regolato dalla valvola di Split).

SSL Concept - Split Mode

Transfer flow through the liner =

Flow through the column + flow through the split line



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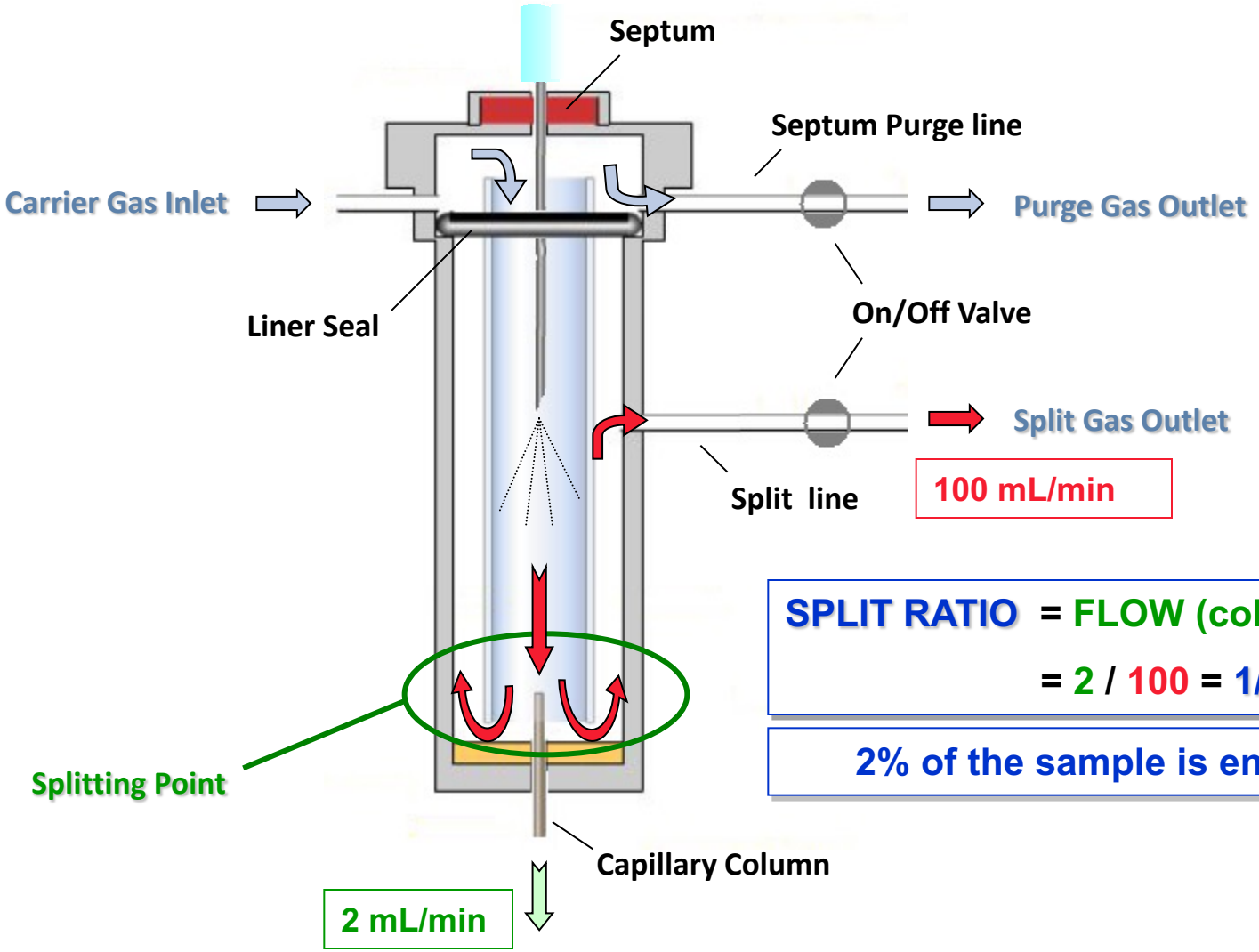
SSL Concept – Split Mode



SPLIT RATIO = **FLOW (column)** / **FLOW (split)**

= **2** / **100** = **1/50**

2% of the sample is entering the column

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INIEZIONE SPLITLESS

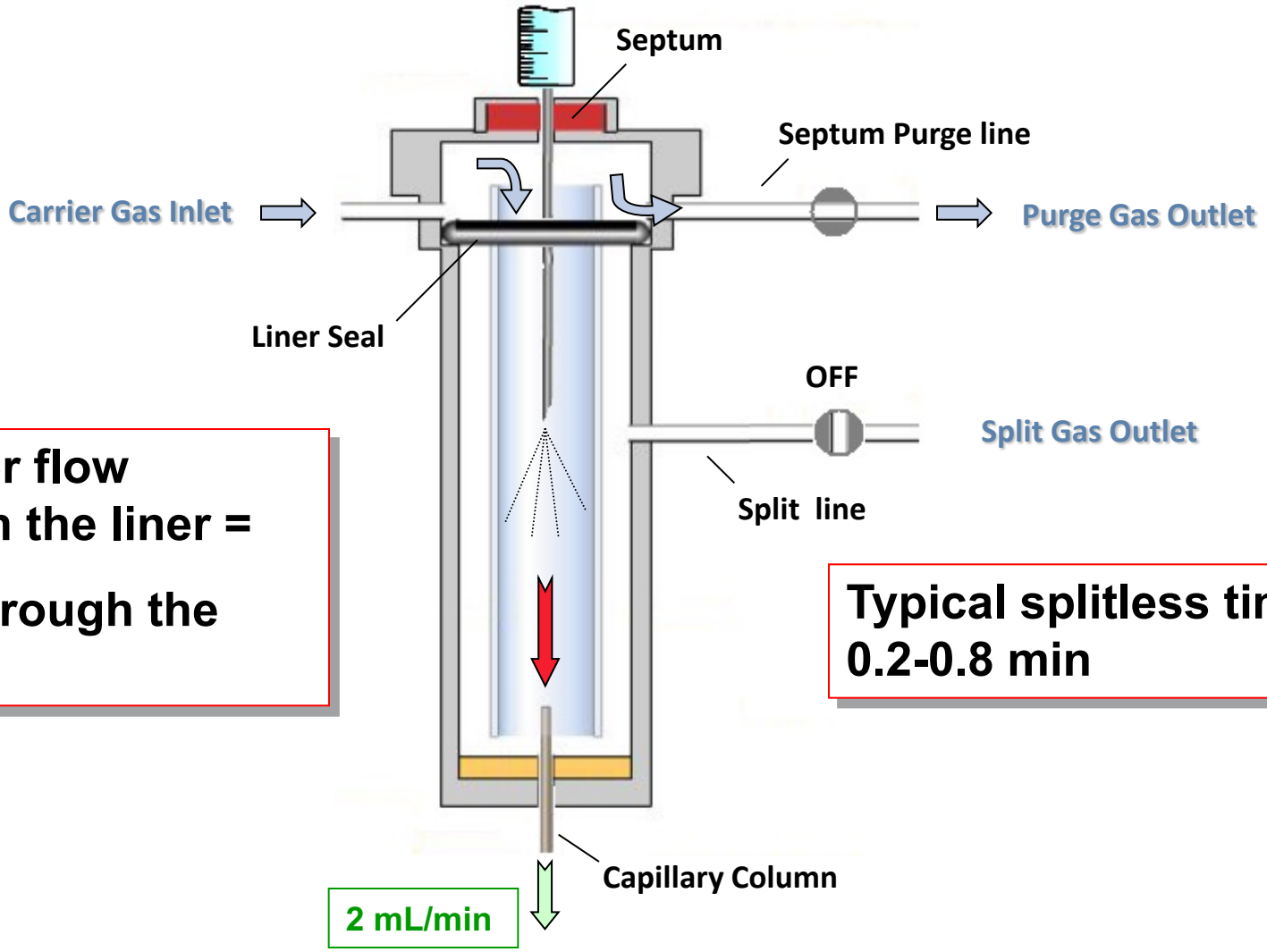
Tutto il campione viene iniettato in colonna.

L'SSL è costituito da una camera di iniezione termostatata a elevata temperatura, in cui il campione evapora rapidamente e si miscela con il gas di trasporto.

Tutto il campione, compreso il solvente, viene introdotto in colonna.

Questo permette di rilevare i composti e le impurezze in tracce (ppb o meno).

SSL Design - Splitless Mode



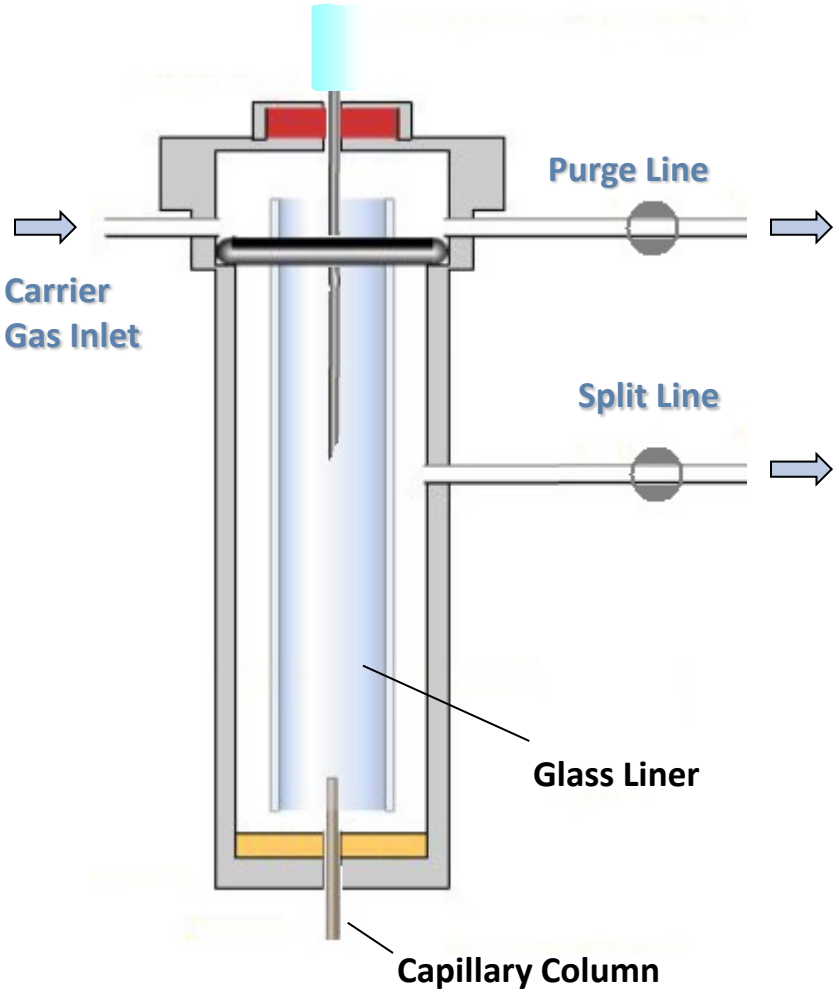
Transfer flow through the liner =

Flow through the column

Typical splitless time 0.2-0.8 min

Split/Splitless Injection – Overload problem

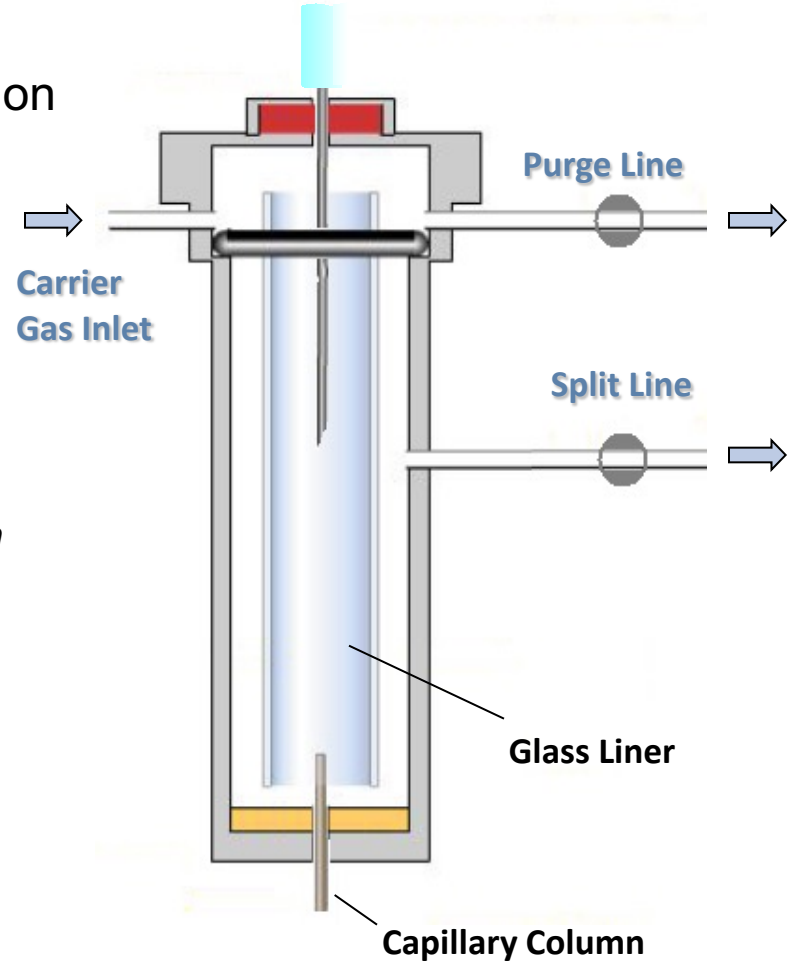
- **Transferred Amount**
  - Risk of sample loss for vaporization chamber overload (splitless mode)
  - Transferred amount related to the split ratio (split mode)
    - *Pressure and flow transients in the inlet during injection may change the actual splitting ratio*



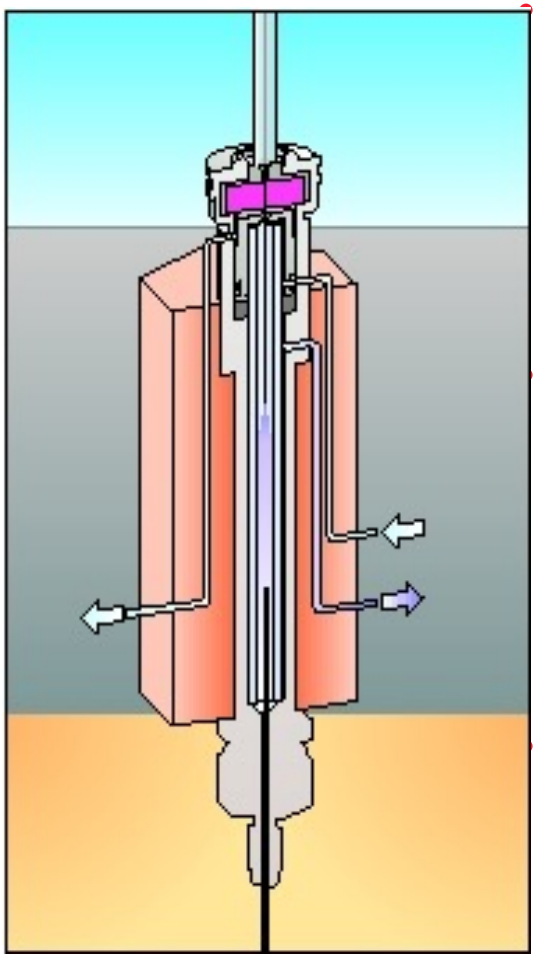
Split/Splitless Injection – Discrimination problem

- **Sample integrity**

  - Discrimination in the syringe needle as a function of the components boiling point
    - *Distillation from a hot needle*
  - Discrimination in the vaporization chamber
    - *Difficult evaporation of high boiling (particularly on packing materials)*
    - *Loss of volatiles (e.g. through the septum purge)*
  - Thermal and catalytical decomposition / Adsorption inside the liner
    - *On packing materials or on by-products deposited in the liner (matrix effect)*



TECNICHE DI INIEZIONE CON INIETTORE SSL



Thermospray (*ad ago caldo o hot needle*)

- Vaporizzazione più uniforme
- Miglior diffusione del campione nel liner
- Svuotamento dell'ago (maggiore sensibilità)
- *Uso di liner vuoti / normali*

Fast Injection *(ad ago freddo)*

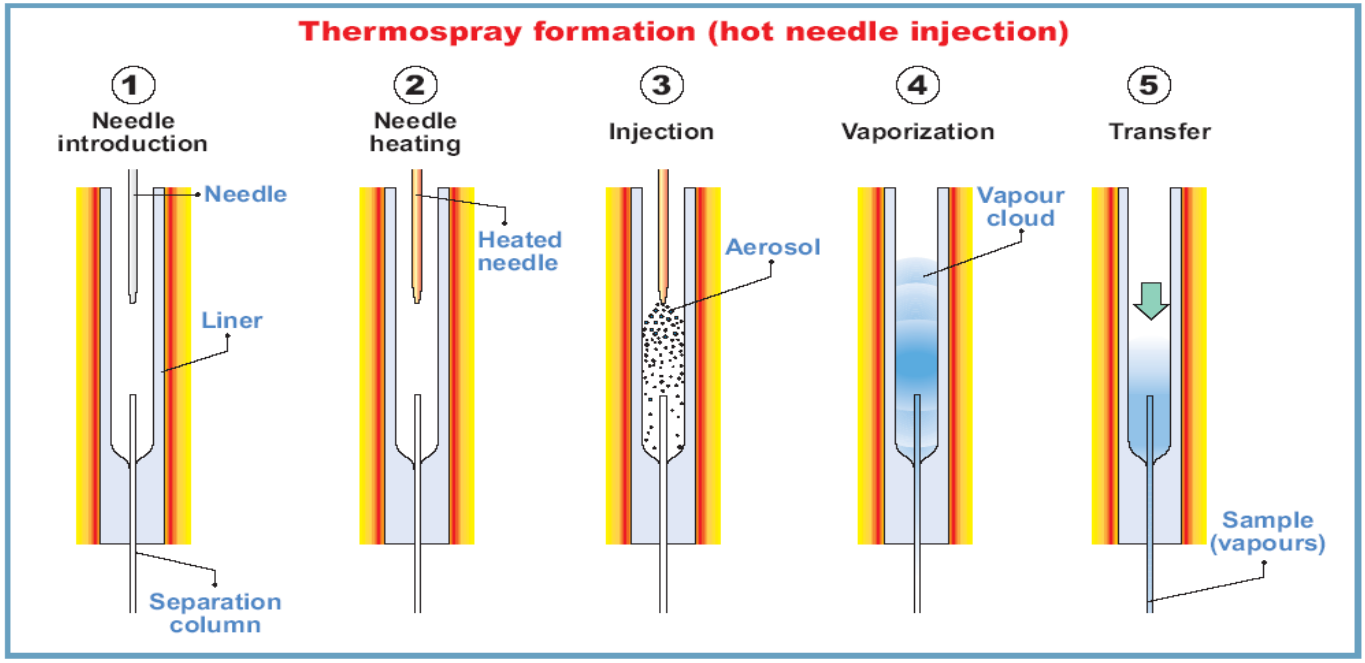
- Veloce vaporizzazione
- Uso di liner con lana di vetro / restrizioni
- Basso volume dell'ago (picchi stretti)
- Ideale per solventi basso bollenti

Pressure Pulse

- Veloce trasferimento in colonna (picchi stretti)
- Riduzione volume di espansione solvente
- Combinabile con Fast o Thermospry Injection

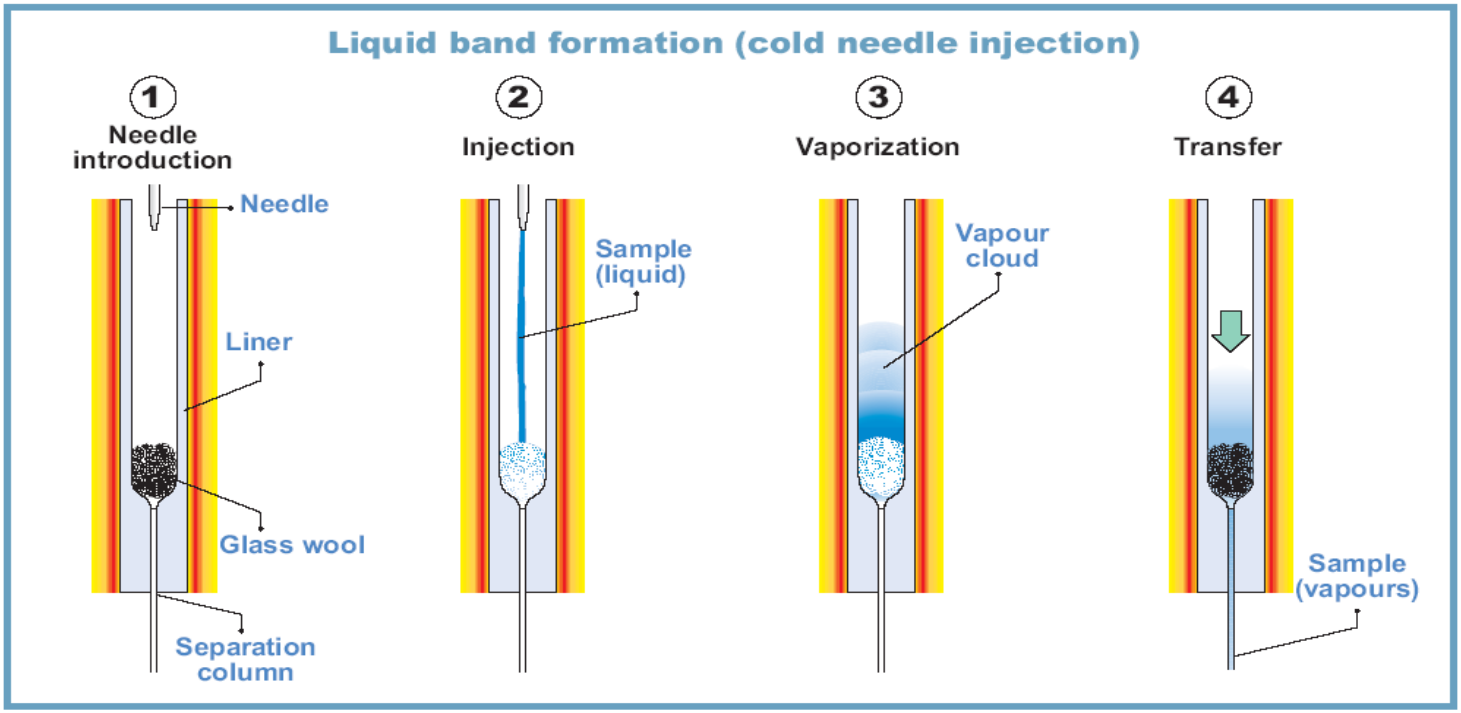
Thermospray Injection (hot needle)

- **Thermospray Formation**
  - A hot needle produces a flash evaporation of the solvent that acts as a propellant for the sample into the needle. It generates nebulisation (aerosol) of liquid expelled from syringe.
  - Evaporation takes place from the small droplets in the dispersed phase. Empty liner.
  - Needle needs to be heated prior to plunger depression. A complete needle penetration and a pre dwell time are required (hot needle technique).



Fast Injection (cold needle)

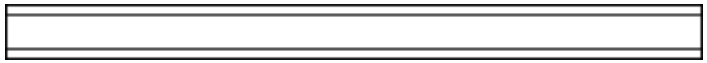
- **Liquid Band Formation**
  - Liquid leaving a cold needle produces a liquid band that travels in the hot chamber (repulsion effect from hot walls)
  - Needle penetrate quickly and for a minimun depth without any dwell time.
  - An obstacle (packing) is necessary to stop the liquid band.
  - Evaporation takes place from a “single droplet” on a surface.



LINER SSL

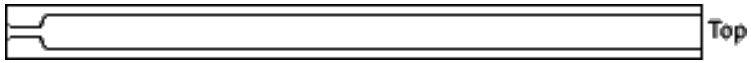
Solitamente è un tubo di vetro, internamente reso inerte mediante un processo di silanizzazione, può contenente lana di vetro, vetro sinterizzato o una o più espansioni che servono ad aumentare la superficie di contatto e così migliorare il trasferimento di calore fra la camera di iniezione e i componenti della miscela favorendone la volatilizzazione.

Split



Diametro interno 1-5 mm

Splitless



LINER SSL e LANA di VETRO

La presenza di lana di vetro, **disattivata**, in un liner SSL, è utile per:

1) Facilitare l'evaporazione del solvente e dei principi attivi iniettati (*cold needle injection in particolare*)

2) Contenere residui indesiderati

LINER SSL e LANA di VETRO

L’aspetto negativo…

…richiede sovente manutenzione a causa della rapidità e facilità con cui si formano siti attivi che possono innescare catalisi o ritenzione di certi principi attivi … pesticidi, fenoli composti organo azotati o comunque tendenzialmente polari.

LINER SSL e LANA di VETRO

In Splitted injection Glasswool will help evaporation;

Reproducibility of peak area's can be < 1%

Precision®
liner

Injection is done in the glasswool plug

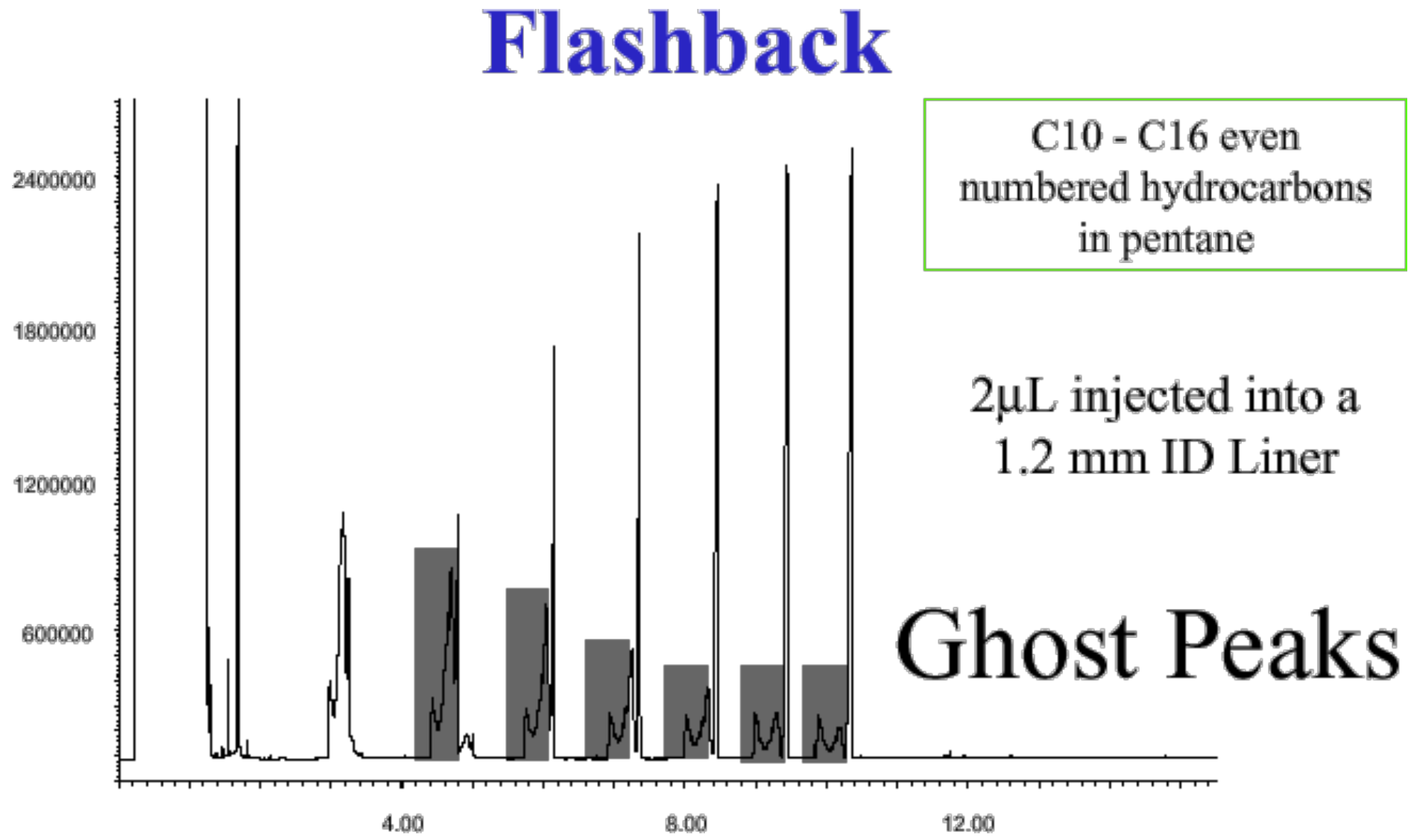
Minimizes the temperature drop caused by solvent evaporation

If sample matrix or components can interact with glasswool, we choose the cyclo-splitter

Cyclosplitter®

Image from Restek Corporation

FLASHBACK EFFECT PROBLEM



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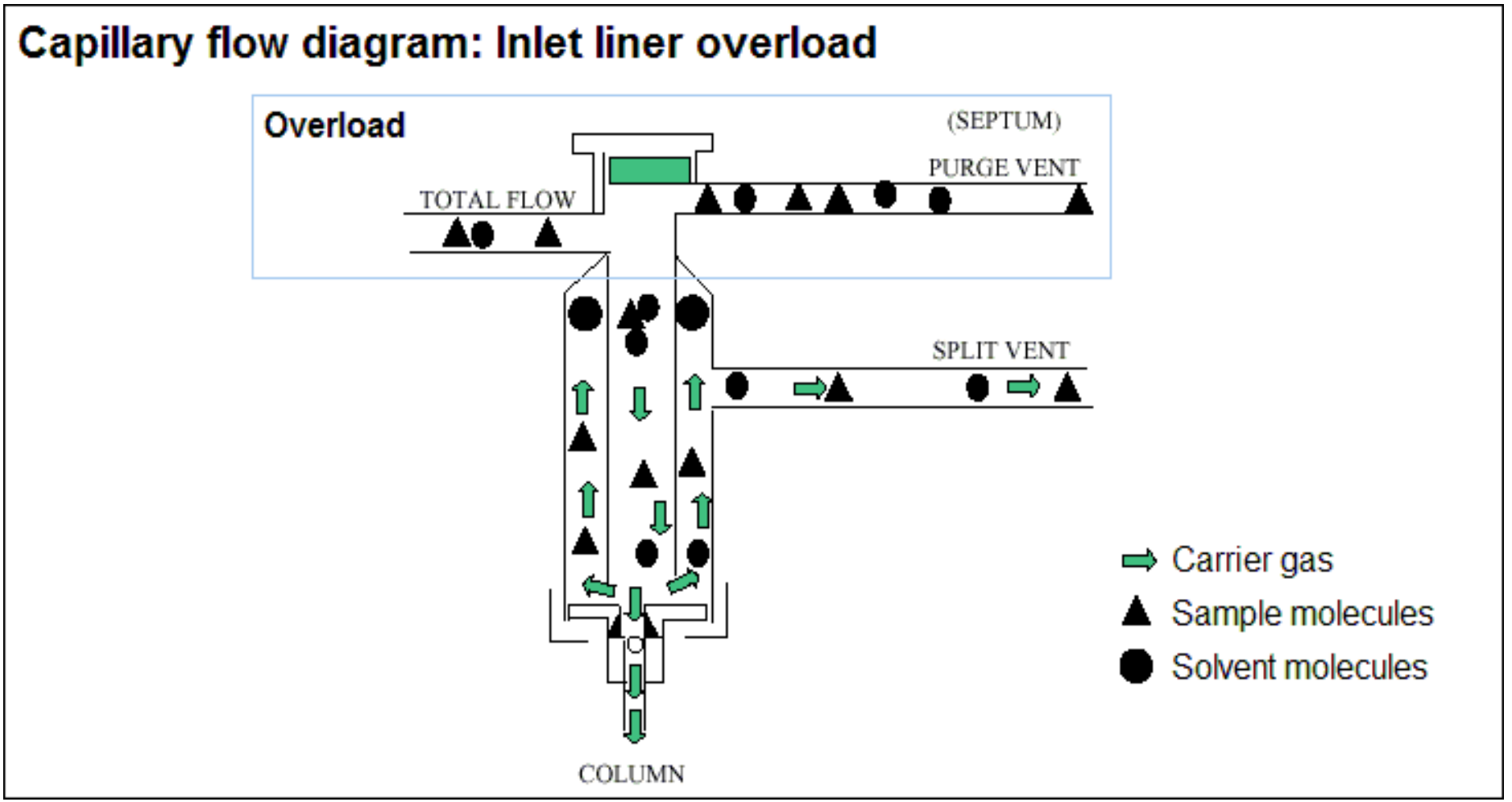
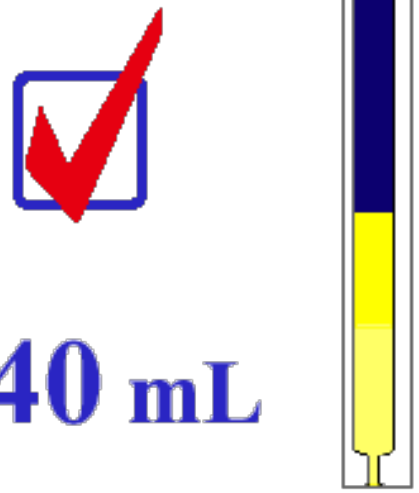
FLASHBACK EFFECT PROBLEM



FLASHBACK EFFECT PROBLEM

Methylene chloride at 250°C and 10 psi inlet pressure will expand to:

1 µL liquid



0.40 mL

3 µL liquid

1.20 mL

FLASHBACK EFFECT PROBLEM

Cause:

Volume occupato dal solvente vaporizzato è maggiore del volume disponibile del liner.

Dove:

Ha luogo esclusivamente in modalità splitless ed in particolare con iniettori di tipo SSL

Soluzioni:

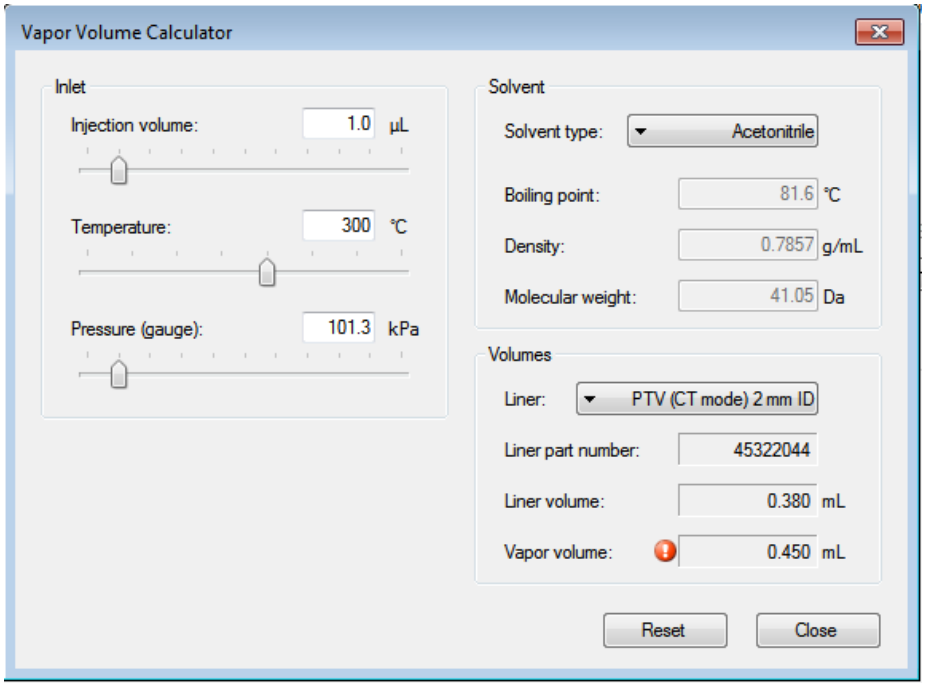
Uso di un liner di volume maggiore
Uso di solventi con minor coeff. di espansione
Uso di un iniettore PTV

VAPOR CALCULATOR vs FLASHBACK EFFECT

VAPOR CALCULATOR software per prevenire il FB effect

- Sample volume expands considerably during vaporization
- The vapor volume produced depends on:

1. Temperature
2. Pressure
3. Injection volume



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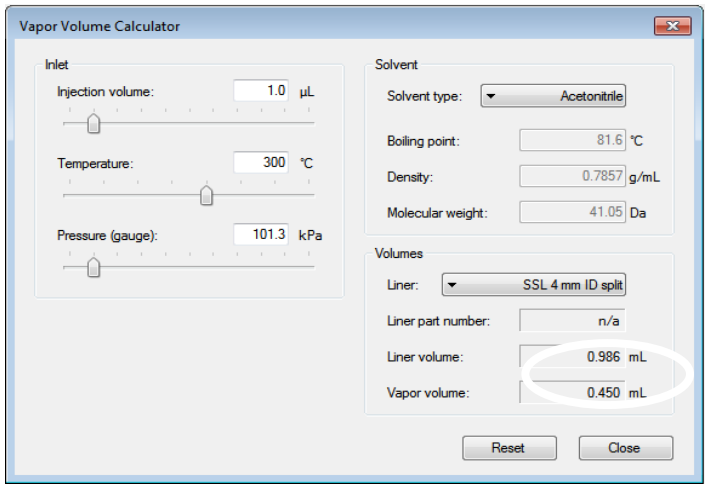
VAPOR CALCULATOR vs FLASHBACK EFFECT

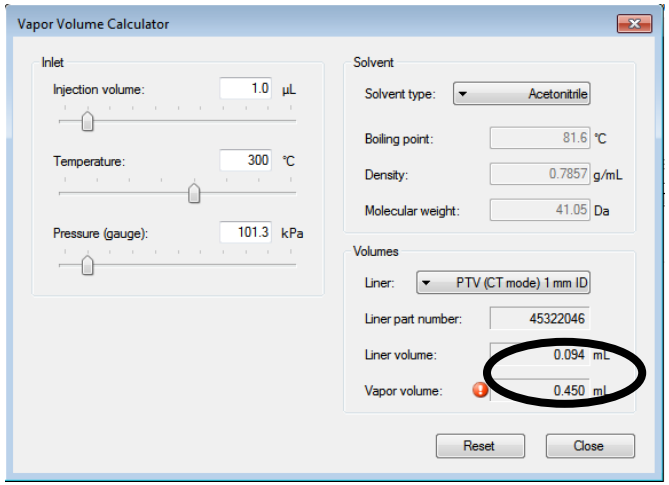
Liner volume

Maximum vapor volume that a liner can contain without loss of analytes

General rule for working in constant temperature

vapor cloud should be retained into the liner (max 50-75% liner volume)





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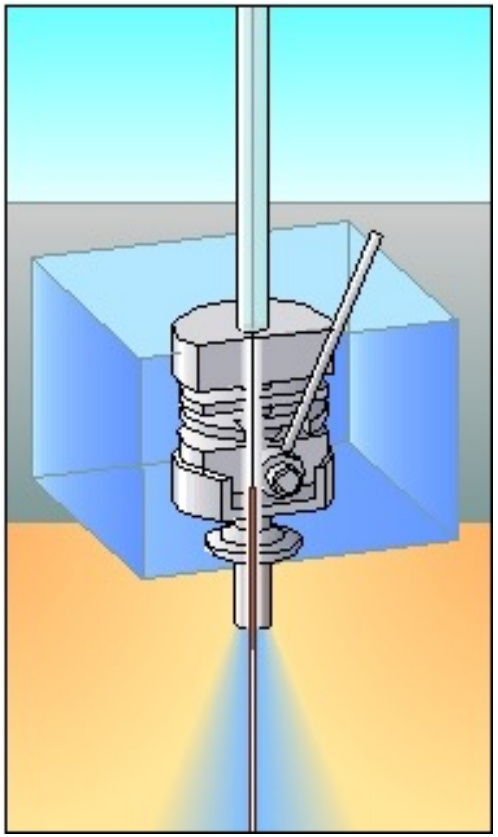
INIETTORE ON-COLUMN



Iniezione direttamente in colonna senza una preventiva vaporizzazione.

Sistema di iniezione detto “a freddo” in modo da prevenire l’evaporazione del solvente nell’ago della siringa.

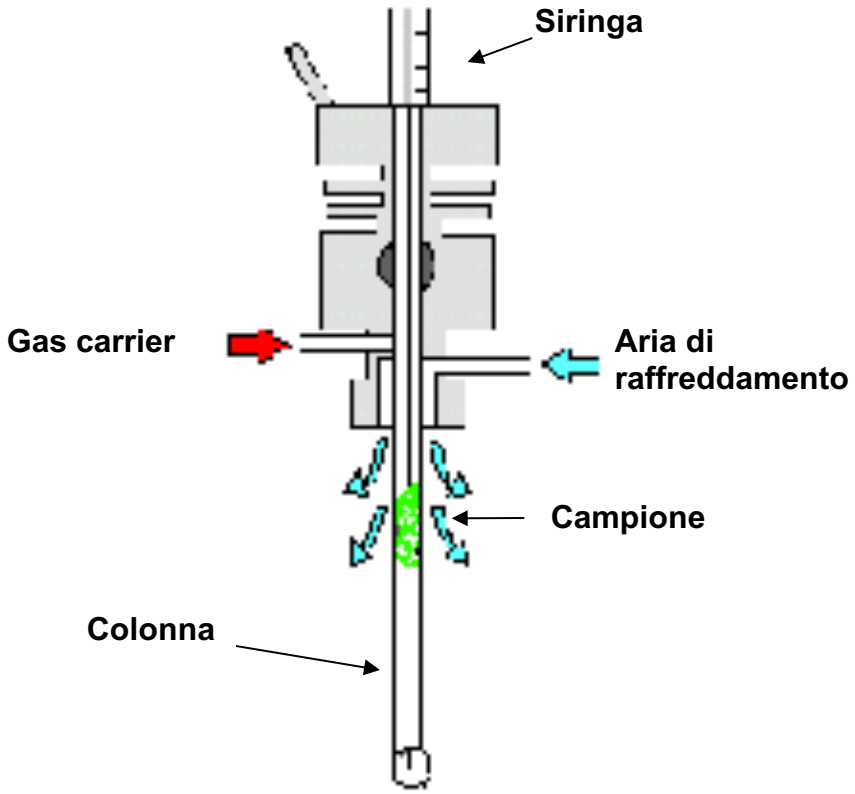
Le siringhe da 1 µl possono iniettare volumi piccoli fino a 0,1 – 0,5 µl in modo sufficientemente riproducibile. Gli aghi usati sono molto sottili, in acciaio o in silice fusa.

INIETTORE ON-COLUMN

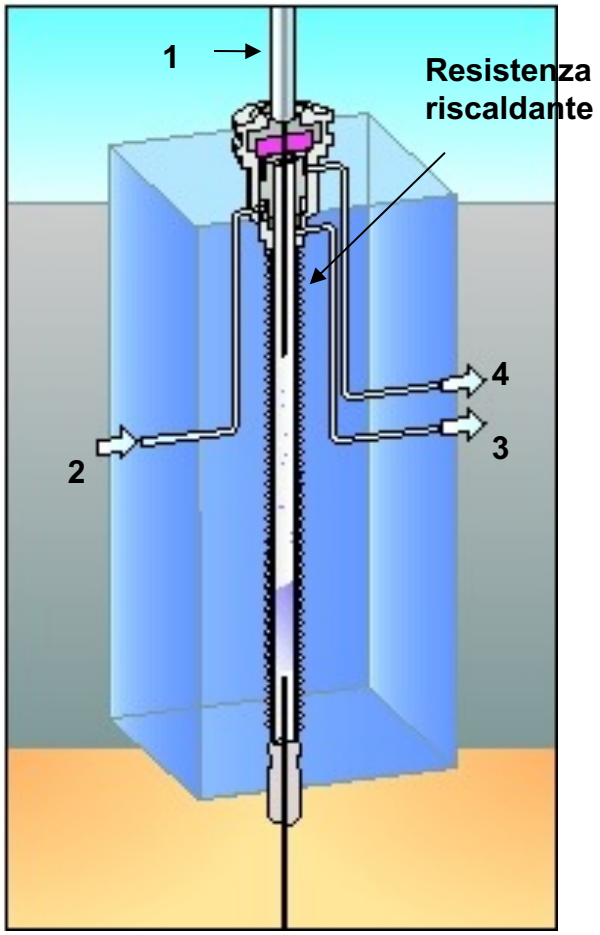
Durante l'iniezione la colonna viene mantenuta ad una temperatura relativamente bassa, molto vicina al p.e. del solvente.

Dal momento che il campione viene iniettato direttamente in colonna si elimina il problema di discriminazione dei componenti altobollenti da quelli bassobollenti.

Indicata per analisi di composti estremamente TERMOLABILI o alto bollenti.



PTV – Programmed Temperature Vaporizer



Iniettore non discriminante a freddo.

Consente di variare la temperatura durante l'iniezione grazie ad una serpentina riscaldante situata intorno al corpo dell'iniettore. I composti presenti nel campione saranno vaporizzati in funzione del p.e.

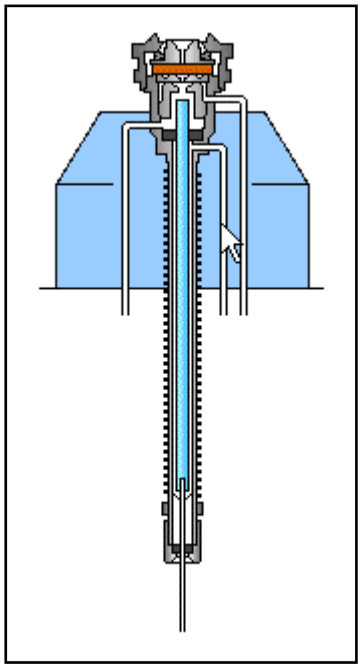
L'intera procedura di riscaldamento dura pochi secondi per garantire un rapido trasferimento del campione in colonna.

1: ingresso campione
2: ingresso gas di trasporto (carrier)
3: linea di splittaggio
4: linea di lavaggio del setto (septum purge)

CARATTERISTICHE DEL PTV

- Possibilità di variare la temperatura (zero discriminazione)
- Possibilità di variare la pressione
- Iniezione LVI (Large Volume Injection) [anche 50 – 100 ul]
- Iniezione on-column
- Modalità Solvent Split
- Fase di pulizia (zero cross contamination)
- Modalità SSL (temperatura costante)
- Elevata versatilità
- Elevata curva di apprendimento

PTV



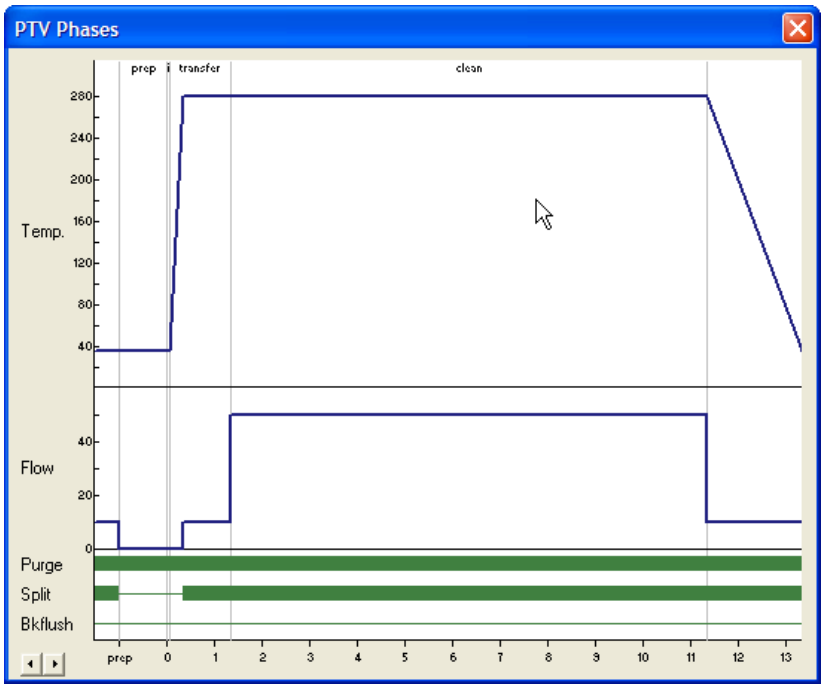
Inlet

Temperature (°C):	35
Split Flow (ml/min):	10
Split Ratio:	10
Splitless Time (min):	0.00
Solv. Valve Temp. (°C):	100

Injection Phases

	Pressure (kPa)	Rate (°C/sec)	Temp. (°C)	Time (min)	Flow (ml/min)
Injection:	70			0.05	50
Evap.:	140	14.5	200	1.00	
Transfer:	210	14.5	280	1.00	
Cleaning:		14.5	280	10.00	50

Show Graph...



Indicata per analisi in tracce di composti anche termolabili

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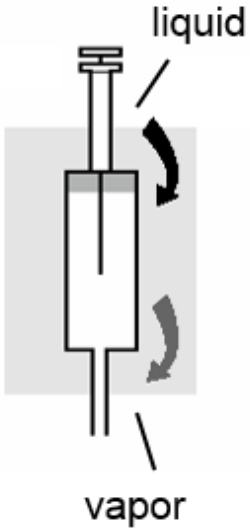
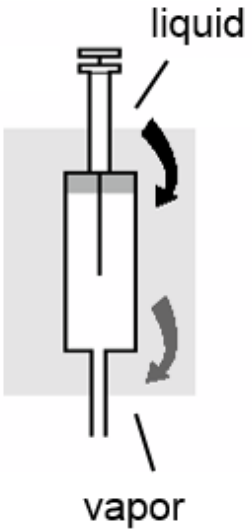
PTV - Splitless Injection Mode



- High sensitivity
- Entire amount of sample is transferred to the column
- 1-2 uL injection volume
- Trace analysis

PTV - Splitless Injection Mode

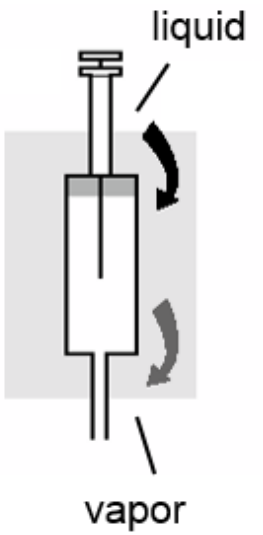
Injection step



Parameters	Typical Setting	Aim
Temperature (PTV initial temperature)	< 10-20°C solvent BP	Minimum vaporization and no loss of low boiling compounds
Time (time before the transfer phase begins)	0,05-0,01 min	
Flow (carrier gas flow through the split line)	Split line is closed	

PTV - Splitless Injection Mode

Transfer step:
sample vaporization and transfer to the column



Parameters	Setting	Aim
Ramp (PTV heating rate to evaporate and transfer the analytes; during the heating ramp the split line is closed)	2,5°C/s	Slow heating rates allows a slower solvent evaporation, therefore the generated vapor cloud is smaller and it can easily be contained by the liner
Temp (maximum temperature for evaporating high boilers)	Last eluting compound BP dependent	The temperature must be high enough to evaporate the high boilers but preventing their degradation
time (time to assure the complete transfer of the components to the column; when the maximum temp is reached the split line is re-opened)	= Splitless time	Splitless time should be long enough to assure the transfer of all the analytes and should be set equal to the transfer time to avoid sample loss due to an early split line opening

PTV - Splitless Injection Mode

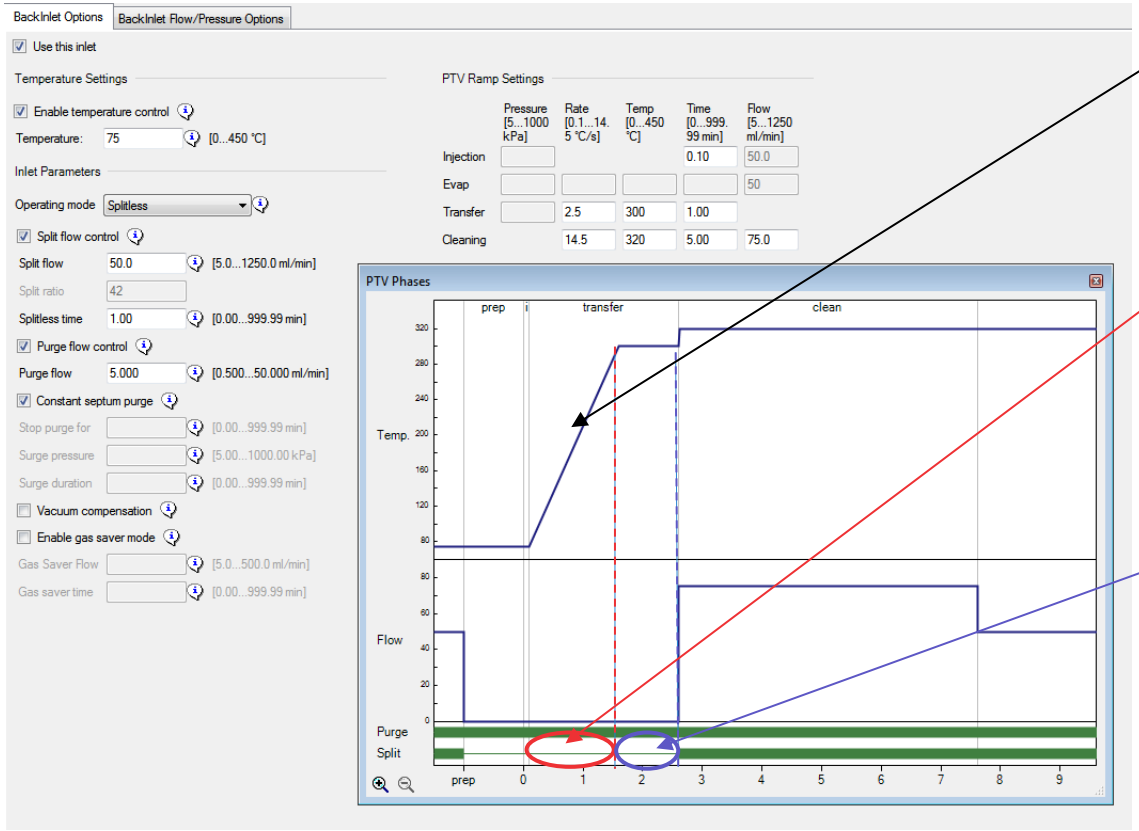
Transfer step:
Split line closure time

split line closure time = ramp time + splitless time

Transfer time = 1,53 min

Ramp time =
from 75°C to 300°C@2,5°C/s

Splitless time = 1 min



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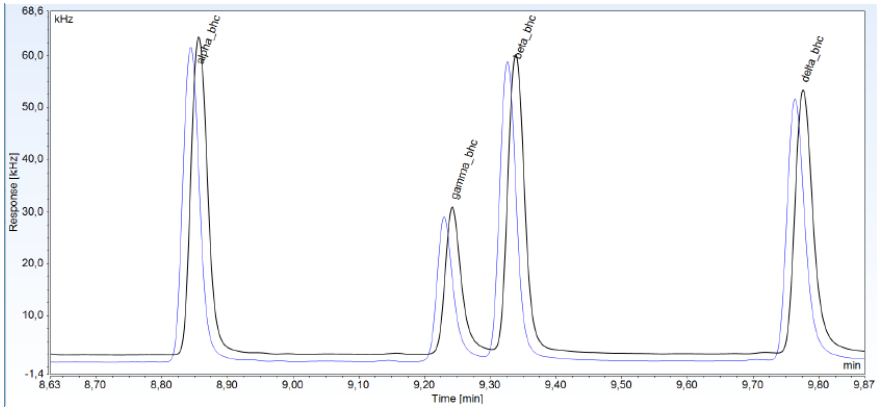
PTV - Splitless Injection Mode

Transfer step:
Split line closure time

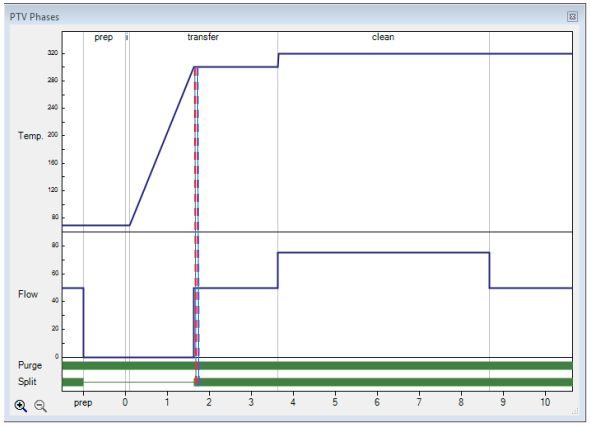
Ramp time from 75°C to 300°C@2,5°C/s = 1.53 min



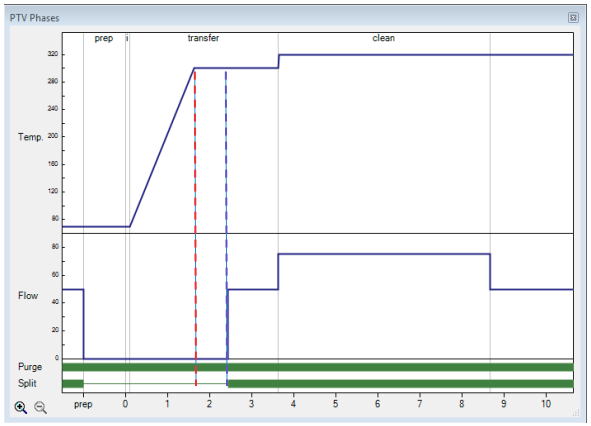
Splitless time =
0 min (black line)

Splitless time =
0,8 min (blue line)

No loss of analytes



Splitless time = 0 min
the split line opens
after transfer steps
(about 1,53 min)

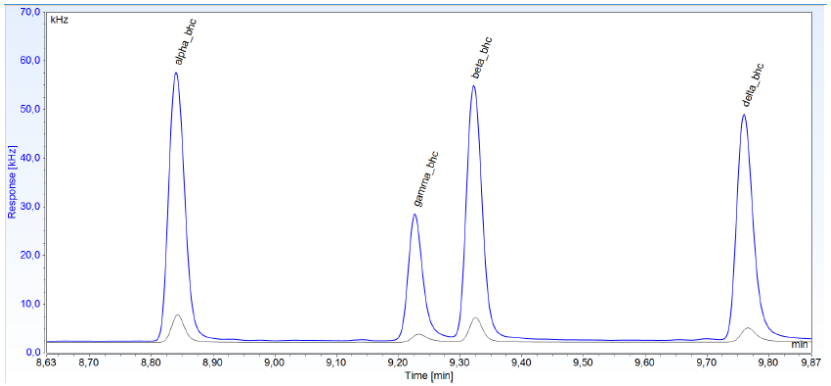


Splitless time = 0,8 min
the split line opens
after transfer steps +
0,8 min
(about 2,33 min)

PTV - Splitless Injection Mode

Transfer step:
Split line closure time

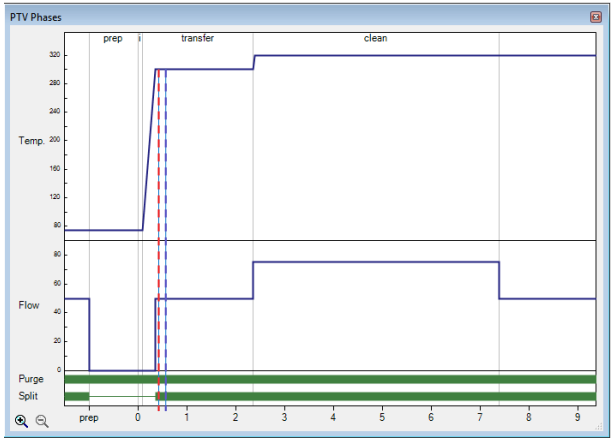
Ramp time from 75°C to 300°C@14,5°C/s = 0.25 min



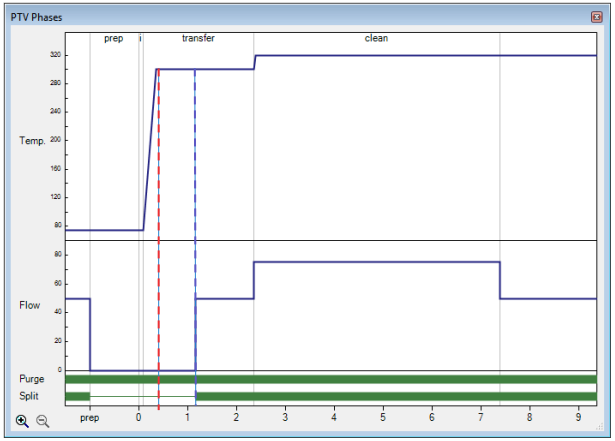
Splitless time =
0 min (black line)

Splitless time =
0,8 min (blue line)

Loss of analytes



Splitless time = 0 min
the split line opens
after transfer steps
(about 0,25 min)



Splitless time = 0,8 min
the split line opens
after transfer steps +
0,8 min
(about 1,05 min)

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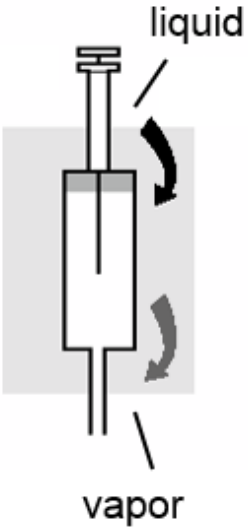
PTV - Splitless Injection Mode

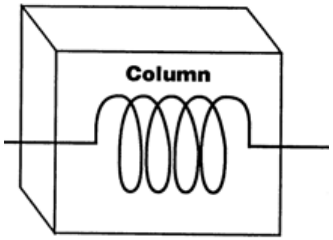
Cleaning step:
Further heating rate to clean the liner from residual vapors and matrix



Parameters	Setting	Aim
Ramp (PTV heating rate to clean the liner)	14,5°C/s	Fast heating ramps allow better evaporation of residual matrix
Temp (maximum temperature for evaporating residuals)	Matrix dependent	Maximum cleaning temperature must be high enough to evaporate all the matrix in order to keep the liner clean
Time (time to assure complete elimination of residuals)	Matrix dependent	High temperature must be held for enough time to assure the evaporation of all residuals in the liner
Flow (carrier gas flow through the split line)	High (70 -80ml/min)	High split rates assure better elimination of residuals from the liner

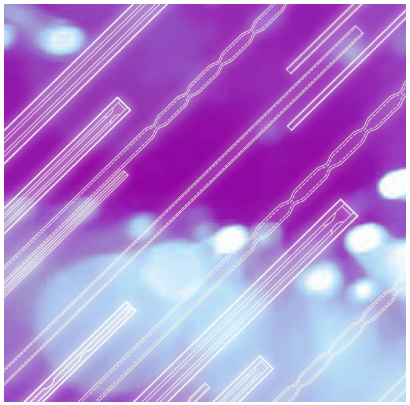
Oven step:

column heating rate to perform chromatographic separation of components



Parameters	Setting	Aim
Initial Oven Temperature (temperature can be set according to the flooding effect)	< solvent BP (if no flooding occurs)	Refocusing the analytes at the top of the column
	> solvent BP (if flooding occurs)	Facilitating evaporation of solvent avoiding its condensation at the top of the column

PTV - Liners For Splitless Injection Mode



Liners enable:

- Correct sample vaporization
- Analyte transfer to the column

Liners can be:

- Straight or baffled
- Empty, packed, sintered
- 1 or 2 mm ID
- Inertness toward the analytes

Liner	ID x L (mm)	Vapor Capacity (µL)	Deactivation	Suggested Conditions	Injection	Suggested Tip Syringe
Metal Liner (P/N 45322044)	2 x 120	380	Siltek®	Low boiling point solvent	Splitless	Cone/Bevel
Quartz Straight Liner (P/N 45322056)	1 x 120	150	Deactivated	High molecular weight compounds	Splitless	Cone/Bevel
3-baffle Liner (P/N 45352062	1 x 120	100	Deactivated	Medium/high boiling point solvent	Splitless	Cone
6-baffle Liner (P/N 453T2120)	2 x 120	350	Siltek®	Medium/high boiling point solvent	Splitless	Cone
5-baffle LinerGold® (P/N 453T2171-UI)	1 x 120	100	Highly Deactivated	Medium/high boiling point solvent, Very sensitive compounds	Splitless	Cone

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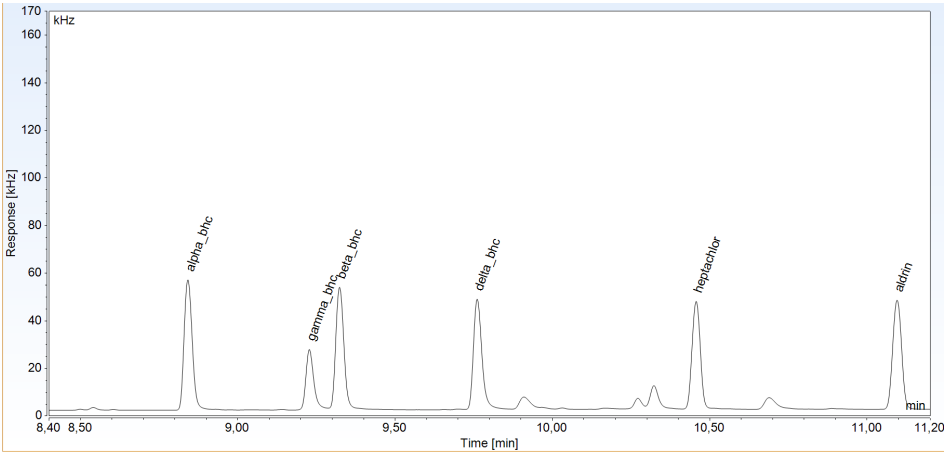
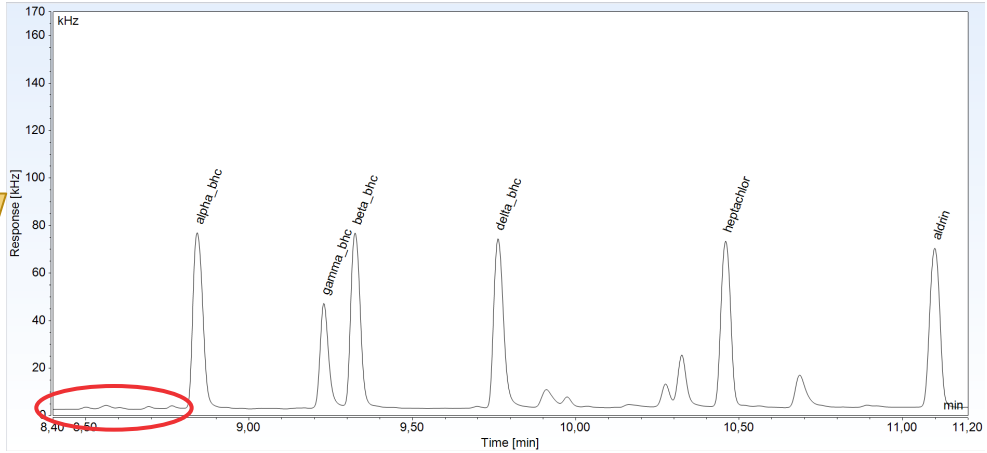
PTV - Splitless Injection: effect of increasing sample volume

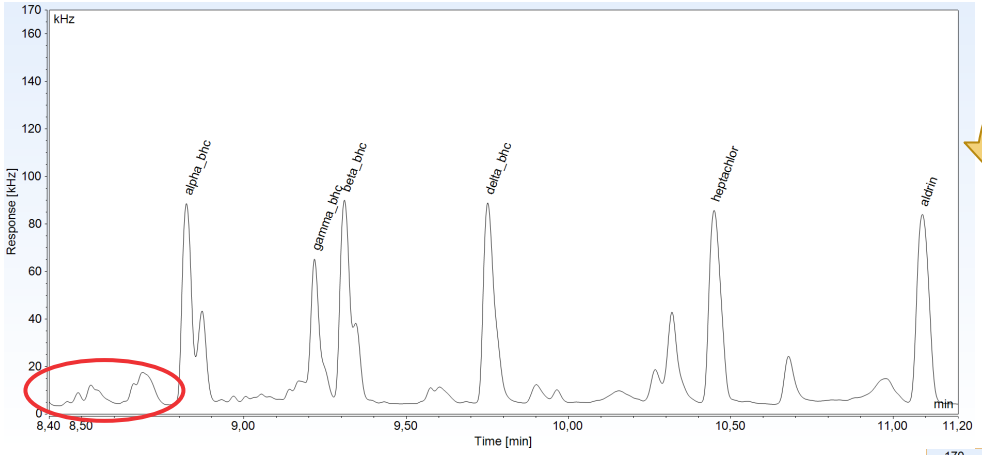


Pesticide analysis using a PTV injector with an ECD
1µL in ACN using a 6-baffle liner

2 µL in ACN
solvent starts to condense at the head of the column causing slight flooding

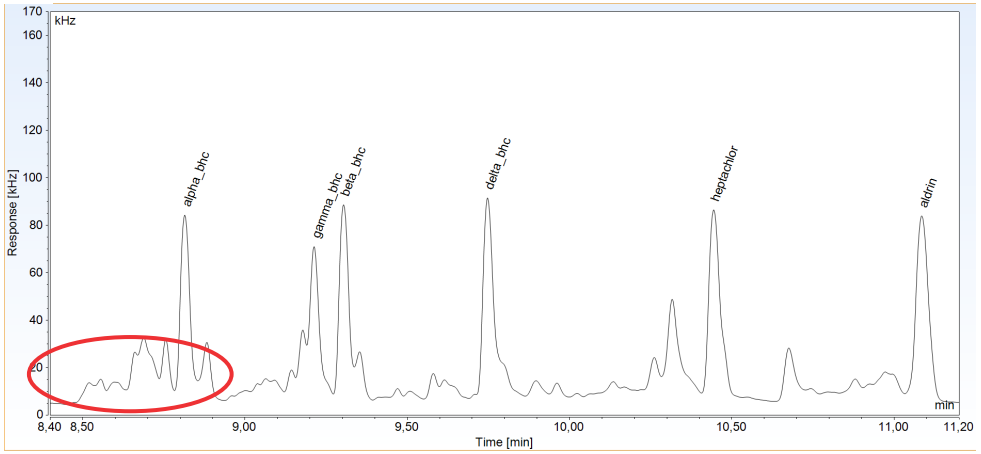


PTV - Splitless Injection: effect of increasing sample volume



4 µL in ACN
flooding effect gets worse ....

.... and worse
5 µL in ACN



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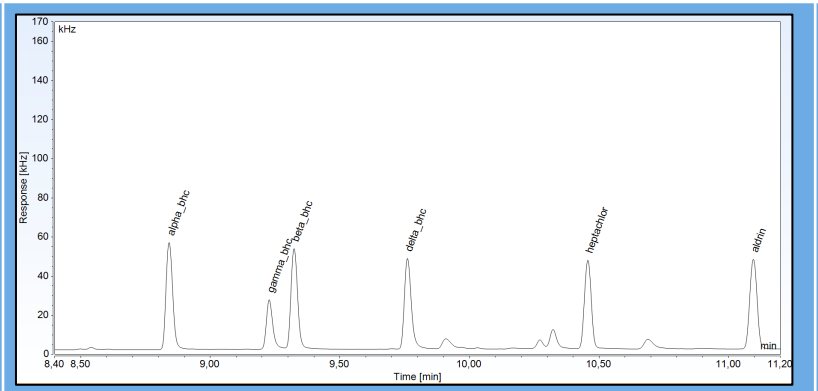
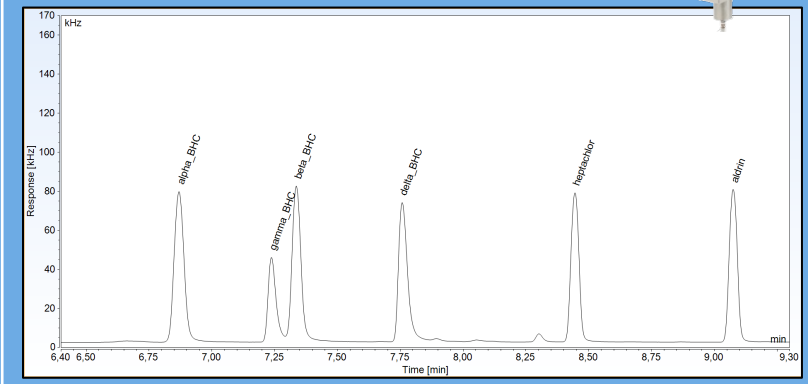
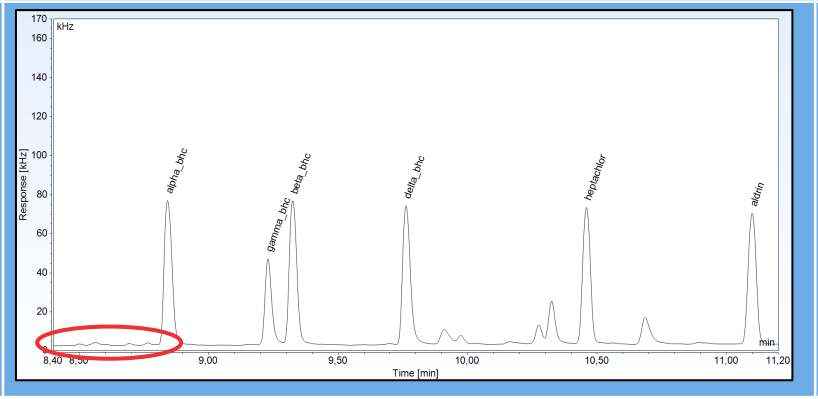
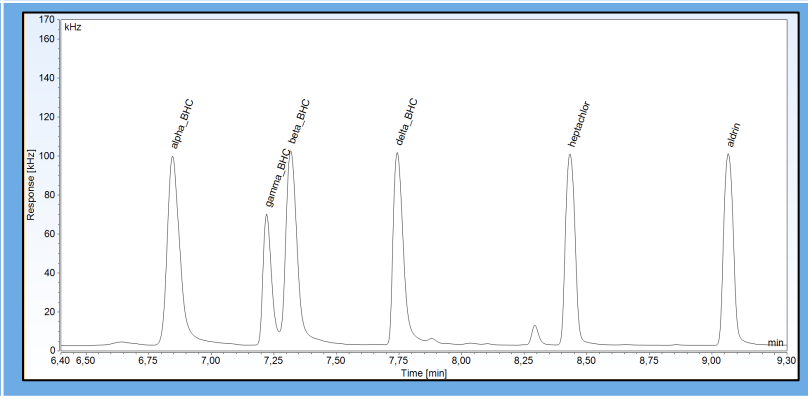
How To Avoid Flooding Effect?

Solution 1:
HIGHER INITIAL OVEN TEMPERATURE

Solution 2:
LARGE VOLUME INJECTION

Solution 1: Increasing Initial Oven Temperature from 100 ° C to **130° C**

Injection volume	100°C	130°C
1 µL ACN (6-baffle liner)		
2 µL ACN (6-baffle liner)		

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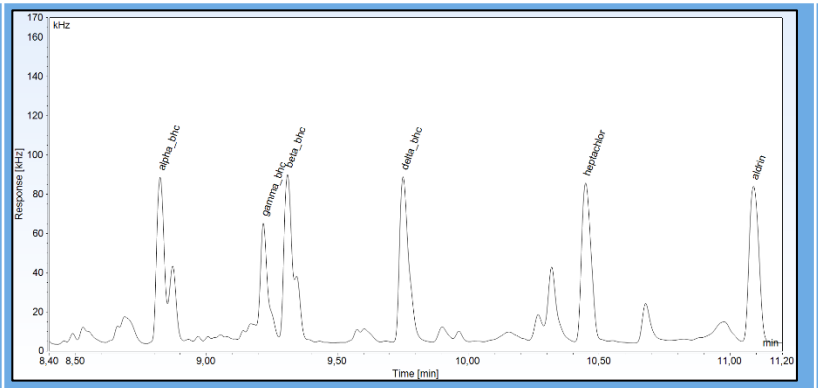
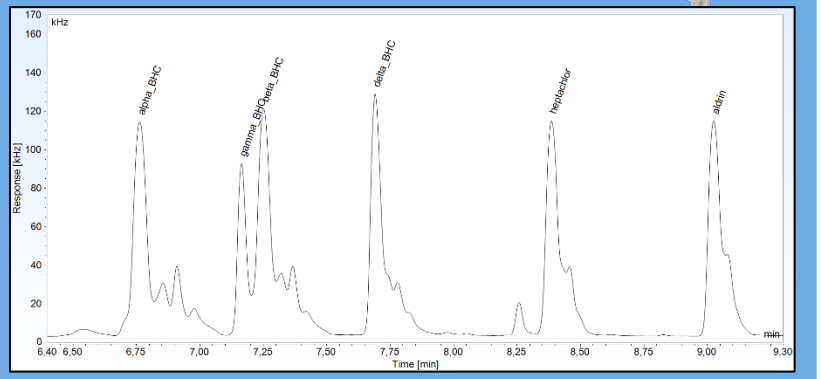
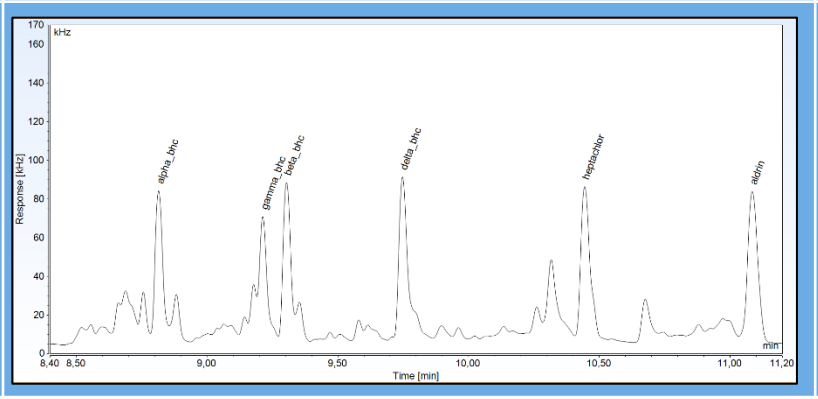
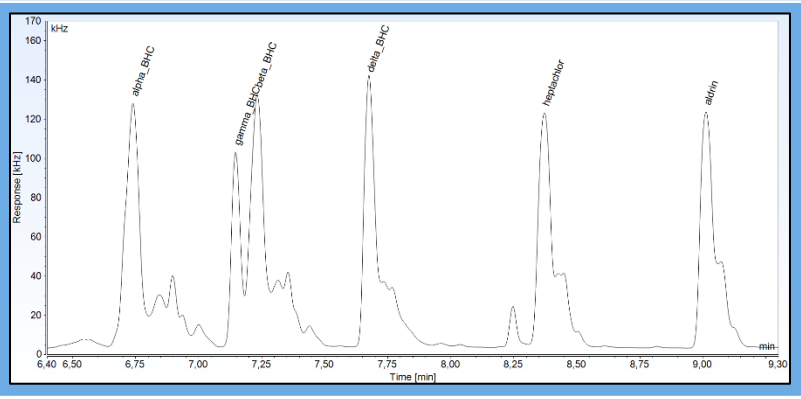
Solution 1: Increasing Initial Oven Temperature from 100 ° C to **130° C**

Injection volume	100°C	130°C
4 µL ACN (6-baffle liner)		
5 µL ACN (6-baffle liner)		

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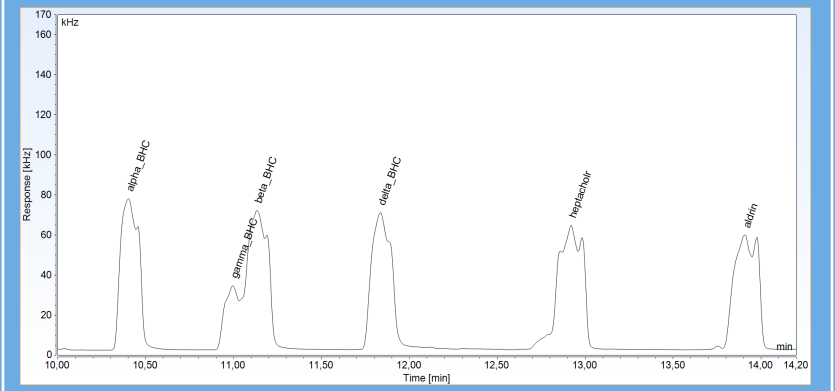
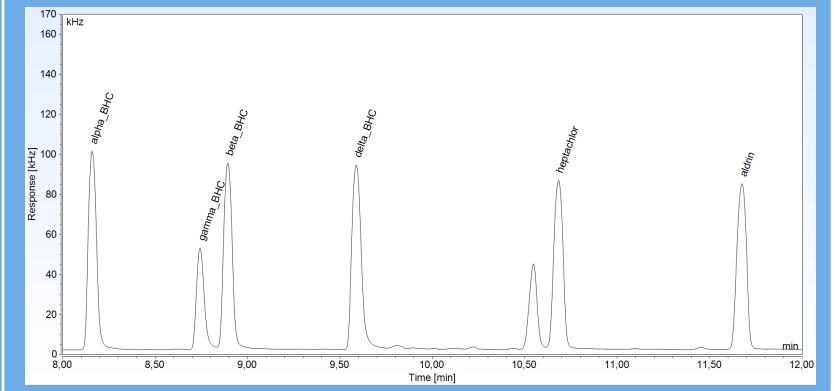
Solution 1: Increasing Initial Oven Temperature from 50 ° C to **100° C**

Injection volume	50°C	100°C
3 µL ISO-OCTANE (6-baffle liner)		

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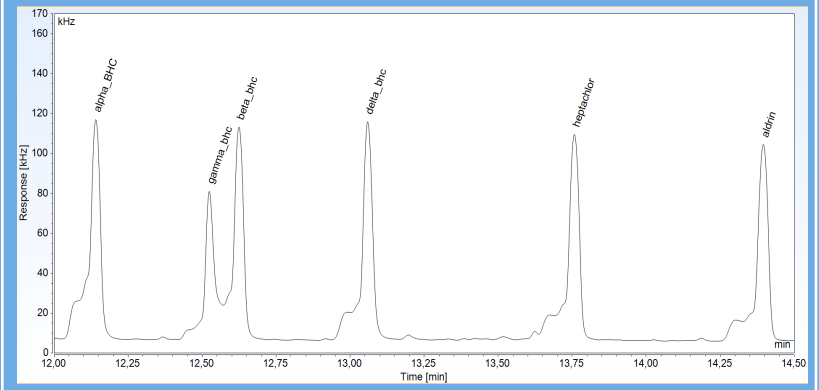
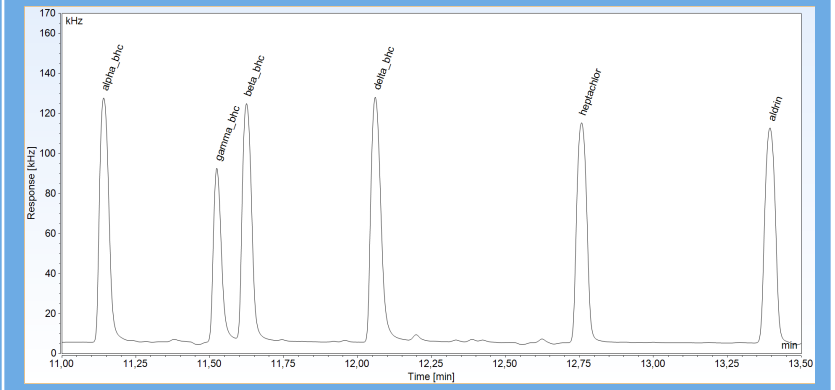
Solution 1: Increasing Initial Oven Temperature from 50 ° C to **65° C**

Injection volume	50°C	65°C
5 µL DCM (6-baffle liner)		

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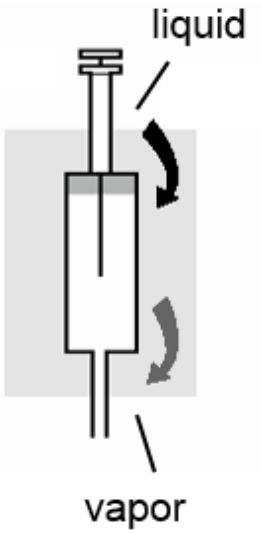
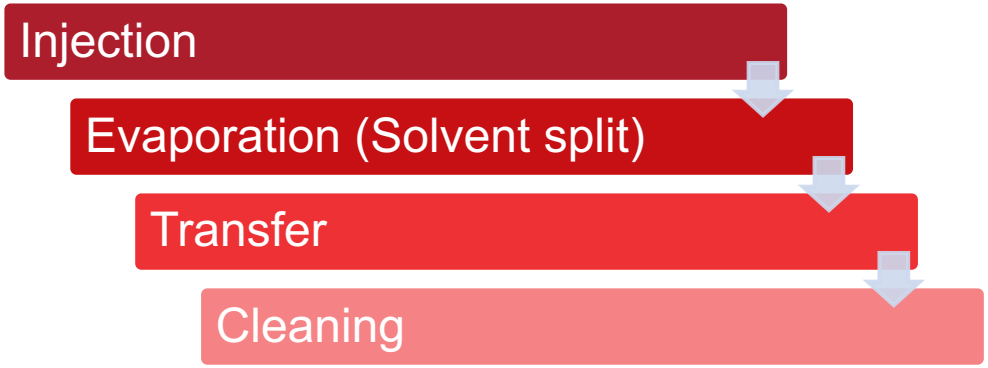
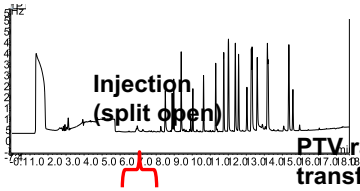
PTV - Large Volume Injection Mode

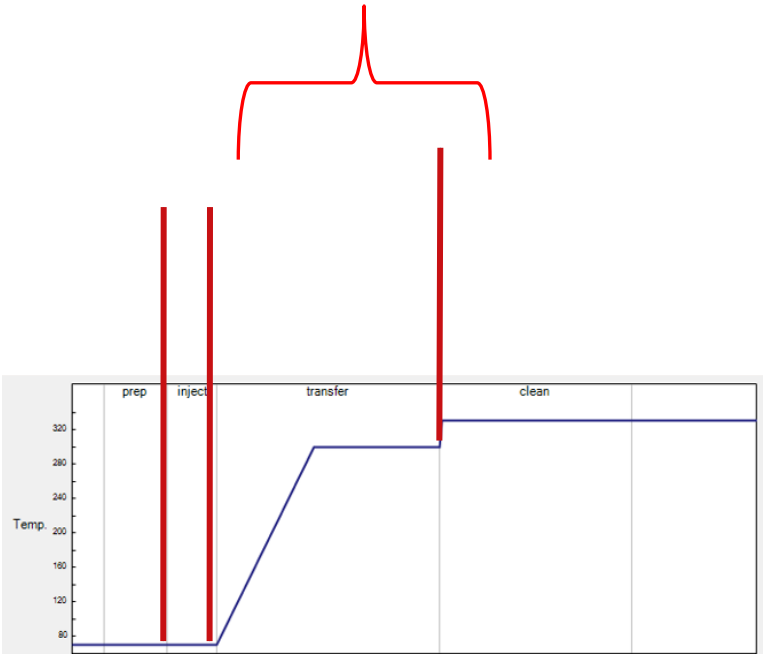


- Higher sensitivity thanks to solvent vent:
  - up to hundreds µL injection volume
  - sample pre-concentration during solvent evaporation
- Efficient transfer of low and high boiling compounds
- Trace and ultra-trace analysis
- Reduced sample preparation steps
- Decreased original sample size and lower solvent consumption and waste



LVI Injection Steps





PTV Phases

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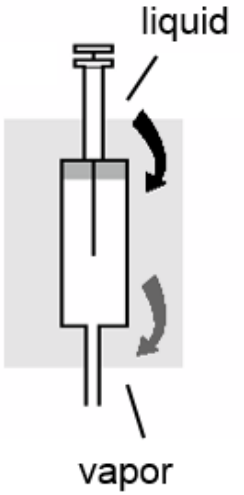
PTV - Large Volume Injection Mode

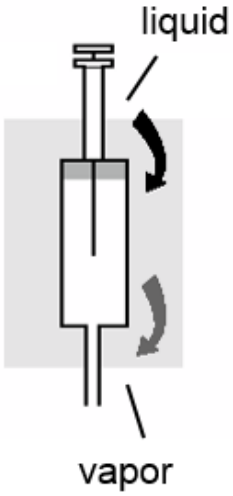
Injection step



Parameters	Typical Setting	Aim
Temperature (PTV initial temperature)	<10 °C solvent BP	Minimum solvent vaporization and no loss of low boiling compounds
Time (time before the transfer phase begins)	0,02-0,10	
Flow (carrier gas flow through the split line)	Low (10-20mL/min)	

PTV - Large Volume Injection Mode

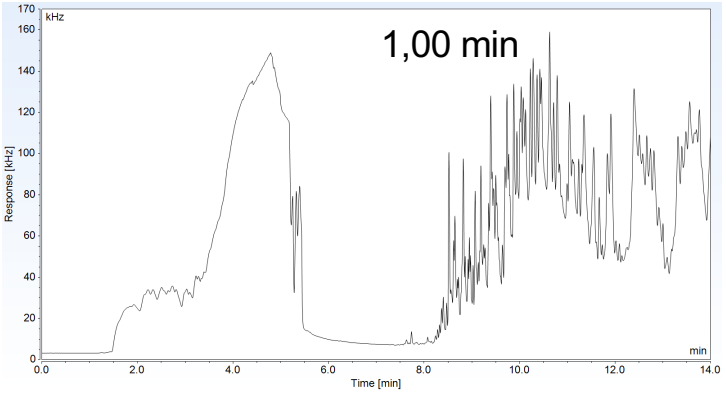
Evaporation (Solvent split)

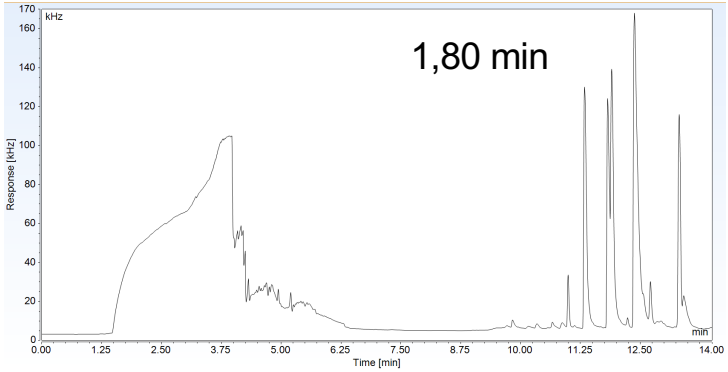


Parameters	Setting	Aim
Ramp **(PTV heating rate to vent the solvent)**	14,5°C/s	Complete vaporization of solvent and minimized loss of volatile compounds
Temp **(maximum allowed temperature for solvent venting)**	Close to the pressure corrected solvent BP	
Time **(time for solvent)**	Solvent volume dependent 0,05 – 2 min	
Flow **(carrier gas flow through the open split line)**	Moderate (50ml/min)	

Vent time

Evaporation step: Vent Time



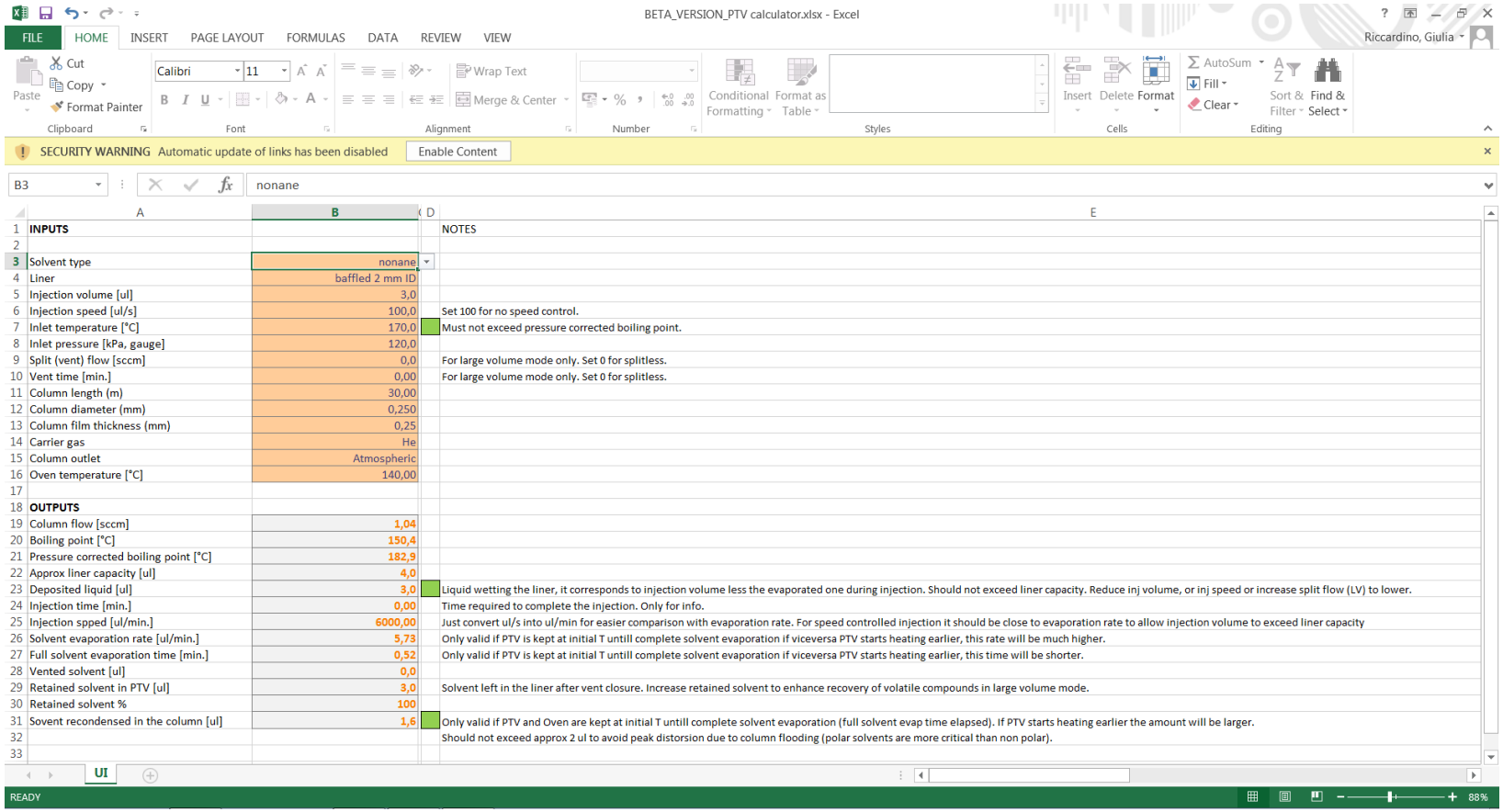


50 uL injection volume solvent vent at different vent times

PTV	**75°C hold 0,02 min** **14,5°C/s to 89 °C hold 1,00 min** **2,5°C/s to 300 °C hold 2 min** **14,5°C/s to 330°C hold 5 min**
SPLIT FLOW	**50mL/min**
SPLITLESS TIME	**2 min**
SEPTUM PURGE	**5 mL/min – CONSTANT**
CARRIER FLOW	**1.2 ml/min**
OVEN	**40°C hold 3 min, 22°C/min to 180 °C, 5°C/min to 270°C, 30°C/min to 320°C hold 3 min**
ECD	**300°C**
INJECTION SPEED	**1 uL/s**
LINER	**sintered**

PTV	**75°C hold 0,02 min** **14,5°C/s to 89 °C hold 1,80 min** **2,5°C/s to 300 °C hold 2 min** **14,5°C/s to 330°C hold 5 min**
SPLIT FLOW	**50mL/min**
SPLITLESS TIME	**2 min**
SEPTUM PURGE	**5 mL/min – CONSTANT**
CARRIER FLOW	**1.2 ml/min**
OVEN	**40°C hold 3 min, 22°C/min to 180 °C, 5°C/min to 270°C, 30°C/min to 320°C hold 3 min**
ECD	**300°C**
INJECTION SPEED	**1 uL/s**
LINER	**sintered**

VERSION Vent Calculator



BETA_VERSION_PTV calculator.xlsx - Excel

SECURITY WARNING Automatic update of links has been disabled — Enable Content

B3: nonane

	A	B	D	E
1	**INPUTS**			NOTES
2				
3	Solvent type	nonane		
4	Liner	baffled 2 mm ID		
5	Injection volume [ul]	3,0		
6	Injection speed [ul/s]	100,0		Set 100 for no speed control.
7	Inlet temperature [°C]	170,0		Must not exceed pressure corrected boiling point.
8	Inlet pressure [kPa, gauge]	120,0		
9	Split (vent) flow [sccm]	0,0		For large volume mode only. Set 0 for splitless.
10	Vent time [min.]	0,00		For large volume mode only. Set 0 for splitless.
11	Column length (m)	30,00		
12	Column diameter (mm)	0,250		
13	Column film thickness (mm)	0,25		
14	Carrier gas	He		
15	Column outlet	Atmospheric		
16	Oven temperature [°C]	140,00		
17				
18	**OUTPUTS**			
19	Column flow [sccm]	1,04		
20	Boiling point [°C]	150,4		
21	Pressure corrected boiling point [°C]	182,9		
22	Approx liner capacity [ul]	4,0		
23	Deposited liquid [ul]	3,0		Liquid wetting the liner, it corresponds to injection volume less the evaporated one during injection. Should not exceed liner capacity. Reduce inj volume, or inj speed or increase split flow (LV) to lower.
24	Injection time [min.]	0,00		Time required to complete the injection. Only for info.
25	Injection spped [ul/min.]	6000,00		Just convert ul/s into ul/min for easier comparison with evaporation rate. For speed controlled injection it should be close to evaporation rate to allow injection volume to exceed liner capacity
26	Solvent evaporation rate [ul/min.]	5,73		Only valid if PTV is kept at initial T untill complete solvent evaporation if viceversa PTV starts heating earlier, this rate will be much higher.
27	Full solvent evaporation time [min.]	0,52		Only valid if PTV is kept at initial T untill complete solvent evaporation if viceversa PTV starts heating earlier, this time will be shorter.
28	Vented solvent [ul]	0,0		
29	Retained solvent in PTV [ul]	3,0		Solvent left in the liner after vent closure. Increase retained solvent to enhance recovery of volatile compounds in large volume mode.
30	Retained solvent %	100		
31	Sovent recondensed in the column [ul]	1,6		Only valid if PTV and Oven are kept at initial T untill complete solvent evaporation (full solvent evap time elapsed). If PTV starts heating earlier the amount will be larger.
32				Should not exceed approx 2 ul to avoid peak distorsion due to column flooding (polar solvents are more critical than non polar).
33				

UI

READY

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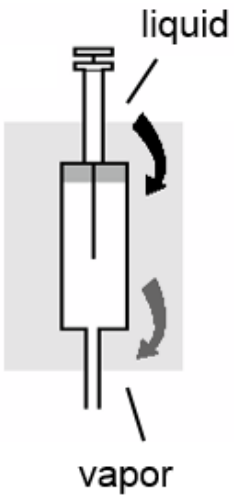
PTV - Large Volume Injection Mode

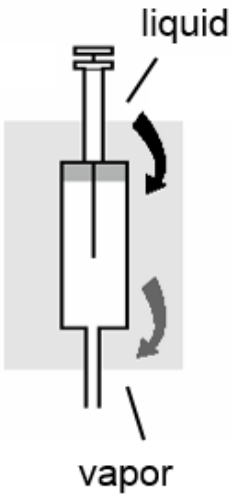
Transfer step: sample vaporization and transfer to the column



Parameters	Setting	Aim
Ramp (PTV heating rate to evaporate and transfer the analytes; during the heating ramp the split line is closed)	2,5°C/s	Slow heating rates allows a slower solvent evaporation, therefore the generated vapor cloud is smaller and it can easily restrained be restrained by the liner
Temp (maximum temperature for evaporating high boilers)	Last eluting compound BP dependent	The temperature must be high enough to evaporate the high boilers but preventing their degradation
time (time to assure the complete transfer of the components to the column; when the maximum temp is reached the split line is re-opened)	= Splitless time	Splitless time should be long enough to assure the transfer of all the analytes and should be set equal to the transfer time to avoid sample loss due to an early split line opening

PTV - Large Volume Injection Mode

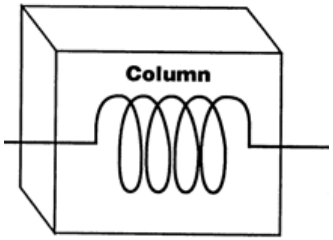
Cleaning step:Further heating rate to clean the liner from residual vapors and matrix



Parameters	Setting	Aim
Ramp (PTV heating rate to clean the liner)	14,5°C/s	Fast heating ramps allow better evaporation of residual matrix
Temp (maximum temperature for evaporating residuals)	Matrix dependent	Maximum cleaning temperature must be high enough to evaporate all the matrix in order to keep the liner clean
Time (time to assure complete elimination of residuals)	Matrix dependent	High temperature must be held for enough time to assure the evaporation of all residuals in the liner
Flow (carrier gas flow through the split line)	High (50 -80ml/min)	High split rates assure better elimination of residuals from the liner

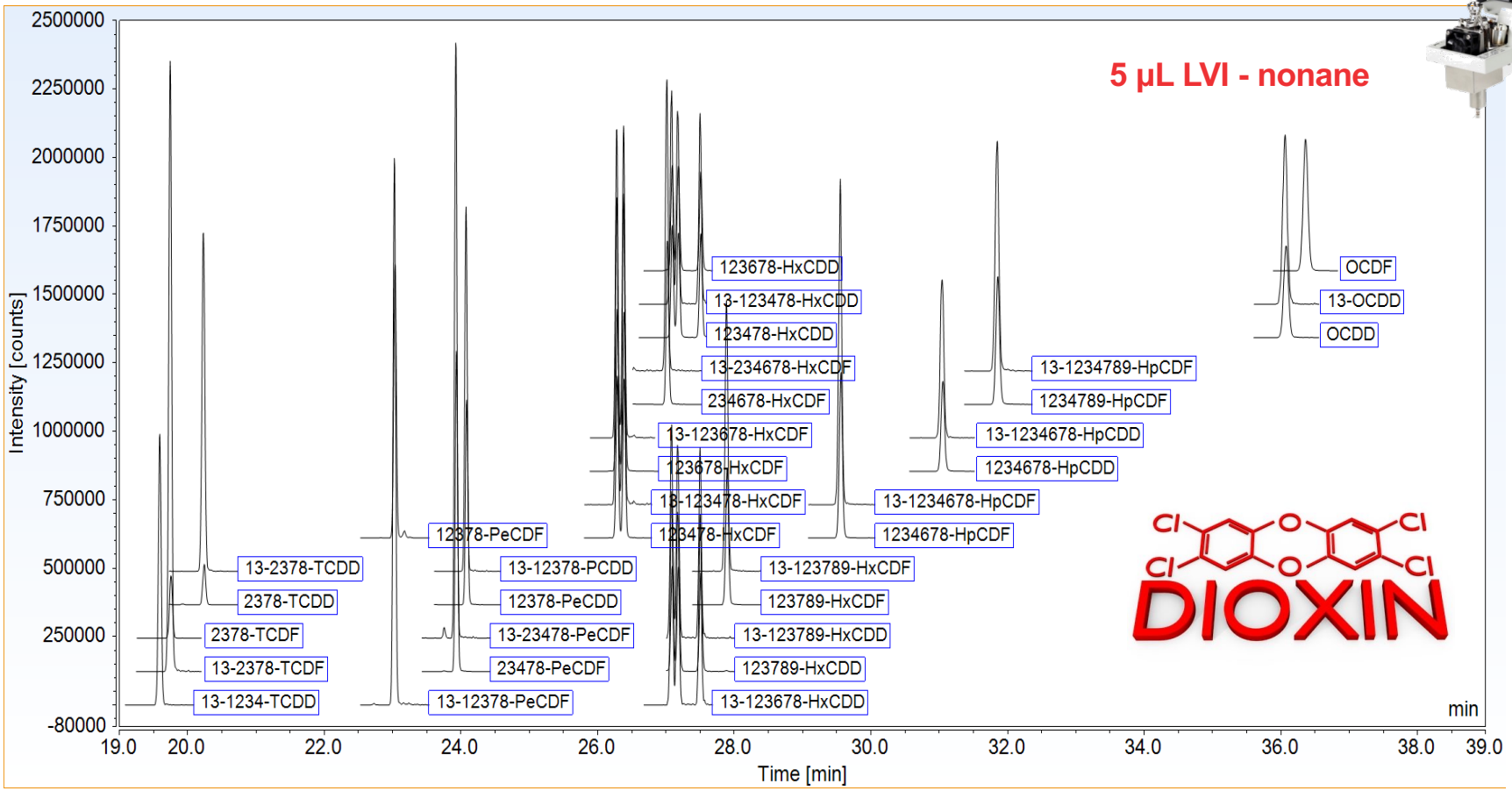
PTV - Large Volume Injection Mode

Oven step:
column heating rate to perform chromatographic separation of components



Parameters	Setting	Aim
Initial Oven Temperature (temperature can be set according to the flooding effect)	< solvent BP (if no flooding occurs)	Refocusing the analytes at the top of the column

PTV - 5 µL LVI – Dioxins Analysis



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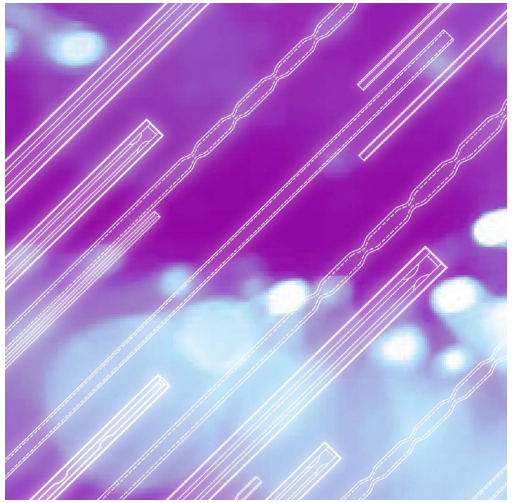
PTV - 5 µL LVI – Dioxins Analysis

LVI Method parametes			
INJECTION VOLUME	5 µL	PTV	130°C hold 0,05 min 14,5°C/s to 300°C hold 2 min 14,5°C/s to 320°C hold 5 min
SOLVENT	NONANE	SPLIT FLOW	50mL/min
ON-COLUMN CONC.	EPA 1613 CS3 5-100 pg (dil.1/10)	SPLITLESS TIME	2 min
LINER (P/N 453T2120)	6-BAFFLES	SEPTUM PURGE	5 mL/min – CONSTANT 1.2 Ml/MIN
COLUMN (P/N 36500525)	TG-DIOXIN 60m-025mm-0,25uL	CARRIER FLOW OVEN	150°C hold 2 min 20°C/min to 250 °C 2,5°C/min to 285°C 10°C/min to 320°C hold 15 min
AQUISITION MODE	MRM		
INSTRUMENT	TSQ8000 EVO triple quad	TRANSFER LINE AND SOURCE TEMPERATURE	280°C

Compounds	%RSD	Compounds	%RSD
13-1234-TCDD	4.55	13-123789-HxCDD	4.94
13-2378-TCDF	4.32	123789-HxCDD	3.41
2378-TCDF	4.49	234678-HxCDF	2.65
13-2378-TCDD	4.53	13-123478-HxCDD	3.38
2378-TCDD	3.25	123478-HxCDD	3.45
13-12378-PeCDF	3.10	13-123678-HxCDD	3.87
12378-PeCDF	2.78	123678-HxCDD	2.13
13-23478-PeCDF	1.93	13-123789-HxCDD	4.94
23478-PeCDF	2.41	123789-HxCDD	3.41
13-12378-PCDD	2.93	13-123789-HxCDF	3.84
12378-PeCDD	2.77	123789-HxCDF	2.65
13-123478-HxCDF	3.37	13-1234678-HpCDF	2.10
123478-HxCDF	2.62	1234678-HpCDF	2.08
13-123678-HxCDF	2.40	13-1234678-HpCDD	1.32
123678-HxCDF	2.25	1234678-HpCDD	1.34
13-234678-HxCDF	2.75	13-1234789-HpCDF	1.66
234678-HxCDF	2.65	1234789-HpCDF	1.30
13-123478-HxCDD	3.38	13-OCDD	3.22
123478-HxCDD	3.45	OCDD	0.67
13-123678-HxCDD	3.87	OCDF	1.72
123678-HxCDD	2.13		

Liners For Large Volume Injection



Liners enable:

- Correct sample vaporization
- Analyte transfer to the column

Liners can be:

- Straight or baffled
- Packed, sintered
- Inertness toward the analytes

Liner	ID x L (mm)	Vapor Capacity (µL)	Deactivation	Suggested Conditions	Injection	Suggested Tip Syringe
6-baffle Liner (P/N 453T2120) 	2 x 120	350	Siltek®	Medium/high boiling point solvent	Splitless Large Volume < 10 µL	Cone
New Sintered Liner (P/N 45352060)  Sintering and deactivation of this liner have been implemented	2 x 120	300	Highly Deactivated	Medium/high boiling point solvent, Very sensitive compounds	Large Volume > 10 µL	Cone/Side Hole

PTV Conclusions

- PTV achieves increasing sensitivity and lowering detection limits thanks to:
  - large volume injection capabilities,
  - reduced inlet discrimination,
  - transfer efficiency,
  - high recovery of both thermolabile and high boiling components.

- Splitless and large volume injection modes can be successfully applied to detect trace amounts of different analytes

- Solvent, injection volume, liner type, temperatures are the main variables involved in PTV analysis and sometimes their optimization can be challenging.

Grazie per l'attenzione