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Liquid Injection Techniques in GC and GC-MS

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Content

Hot split and splitless injection

- Important parameters
- Large volume injection
- Choosing the liner and other consumables
- Maintenance

Programmable temperature vaporization

- Important parameters
- Large volume injection
- Choosing the liner and other consumables
- Maintenance

Backflush injection

- Important parameters

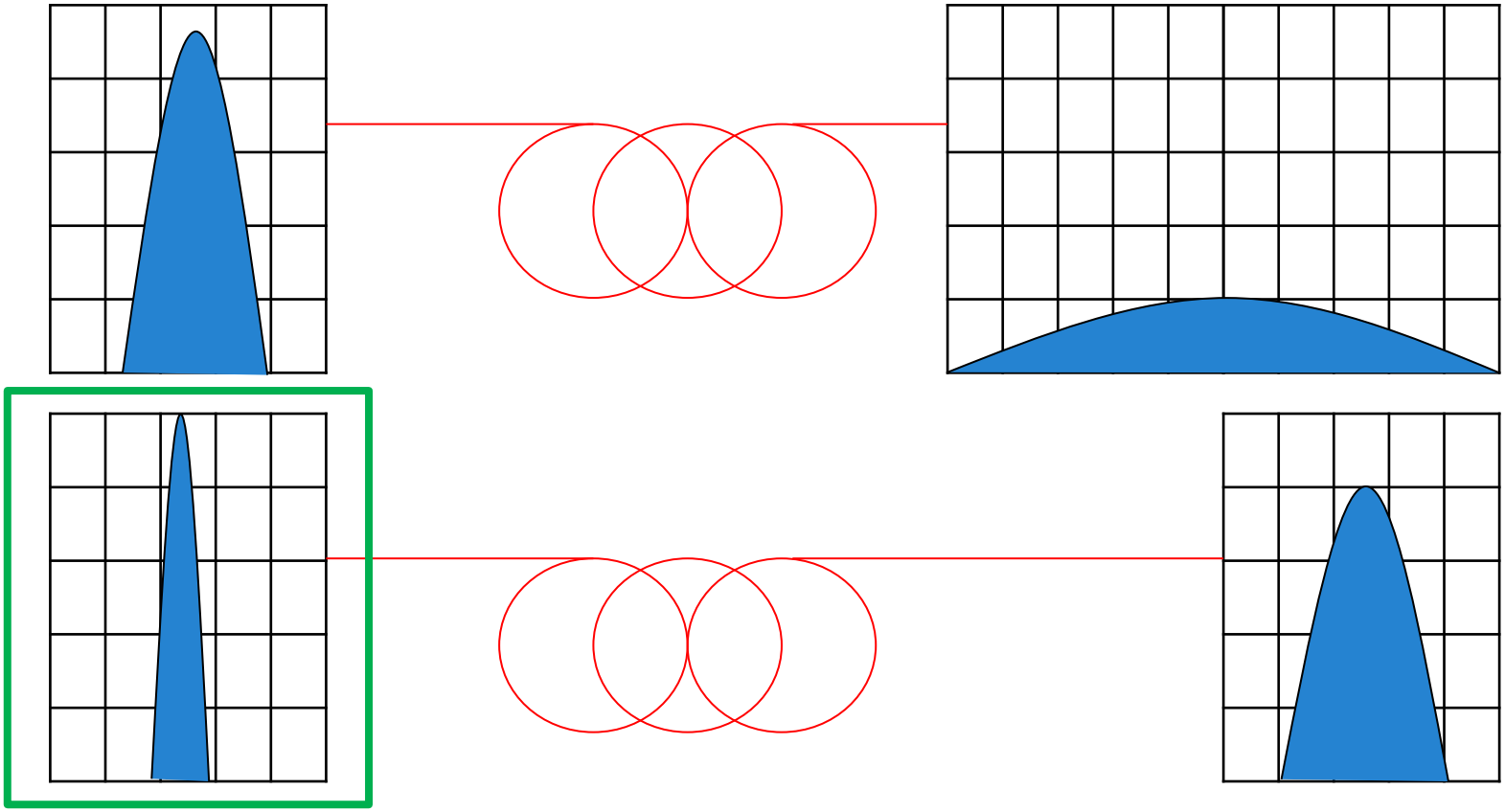
Common issues and solutions

Overview of resources

Hot Split/Splitless Injection

- Important parameters
- Large volume
- Liners and other consumables
- Maintenance

Chromatographic Peaks: Peak Broadening

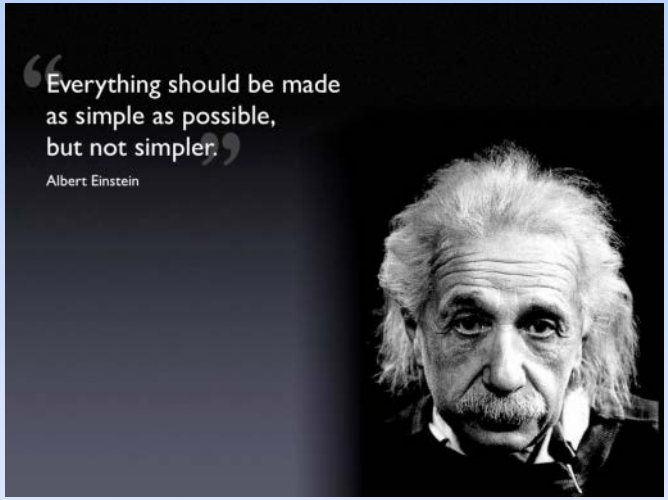


Initial peak width

Final peak width

How to Get a Good Initial Peak Shape?

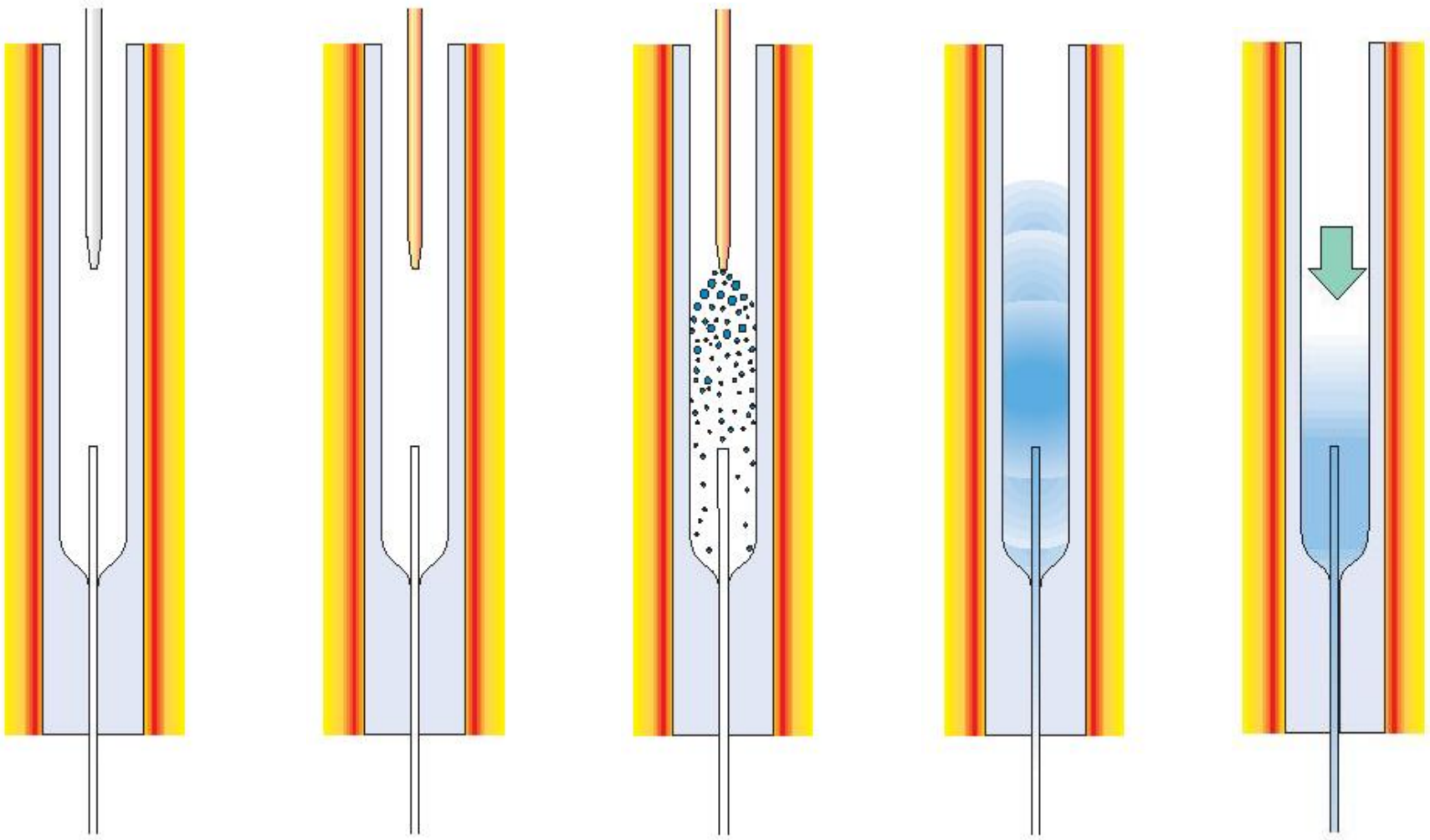
- **SPLIT injection**
  - Splitflow is ON
  - High flow inside the injector
  - Very rapid transfer to the column and no overloading of the phase
  - Usually a good and sharp peak is obtained



- **SPLITLESS injection**
  - Splitflow is off
  - Low flow inside the liner
  - Sample transfer is slow, which could lead to broad peaks or double peaks
  - Solvent trapping by setting the GC oven low
  - Polarity of the solvent should match the polarity of the phase

Liquid Band Formation or “Fast Injection”

Thermospray



Important Instrument Parameters for *Splitless* Injection

- Injection volume
  - **Typical volume is 1-2 µl**
  - Dependent on
    - Solvent vapour
    - Liner volume
    - Pressure
  - Calculation inside GC software
  - TIP: For more polar solvent
    A surge (Or elevated) pressure during injection will allow a larger injection volume

- Injection temperature
  - **Typical temperature is 250 - 300 C**
  - Dependent on analytes and solvent

- Initial GC temperature
  - **Typical 10 to 20C below corrected boiling point solvent**
  - **Please note:** Some column types, e.g. WAX have a minimal temperature (Package)

- Splitless time
  - **Typical time is 1 minute**
  - Analytes need to be vaporized and transferred to GC column
  - After splitless time: Split valve can be opened and liner can be cleaned;
    typical split flow 25 50ml/min

- **Ask yourself these questions**

  - Is the vapor volume not bigger as the liner volume?
  - Am I transferring the sample completely to the GC?
  - How is the peakshape?

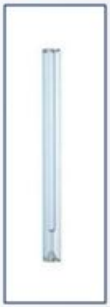
Liner Choice

Liner Choice: Types and Recommendations (1)

- **SPLIT injection**:
  - Straight liner
  - Can be with or without packing
  - In case of headspace a narrow liner is preferred

- We have a selection tool for you

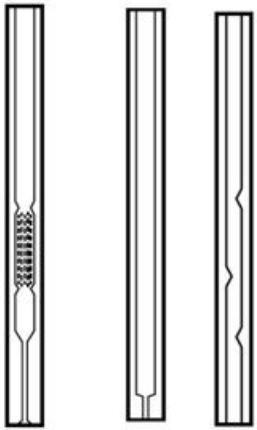
- **SPLITLESS injection; thermospray**
  - Typically no packing inside the liner
  - Narrow end at the bottom of the liner



  - Pro's: No packing
  - No packing= Less active sites
  - Con's: More high boiler discrimination due to heating of the syringe

Liner Choice: Types and Recommendations (2)

- **SPLITLESS injection:**
  - **Liquid band formation**



- **Thermo Scientific™ LinerGOLD™ offer good inertness**

- **1: Tapered liner**
  - Helps to contain the vapor cloud away from the bottom seal
  - Also in double taper (Top and bottom) to keep the vapor away from septum purge
  - Pro: Reduces activity and can be cleaned
- **2: Packed liner**
  - Liner with packing, mostly glasswool, can also be carbofrit
  - More surface area; can contain more vapor
  - Pro: More volume can be injected, and particles are retained
  - Con: Can be slightly more active, dependent on packing
- **3: Baffled/ cyclo liner/ laminary cup**
  - Liner with increased surface area for larger volume
  - Better for high boilers >C26
  - Baffles: Create faster flow inside liner
  - Laminary cup: Bigger volume

Need advise for your application?
We are here: Ask us at analyze.eu@thermofisher.com or check out the Liner Selection guide

The Septum of the Injector

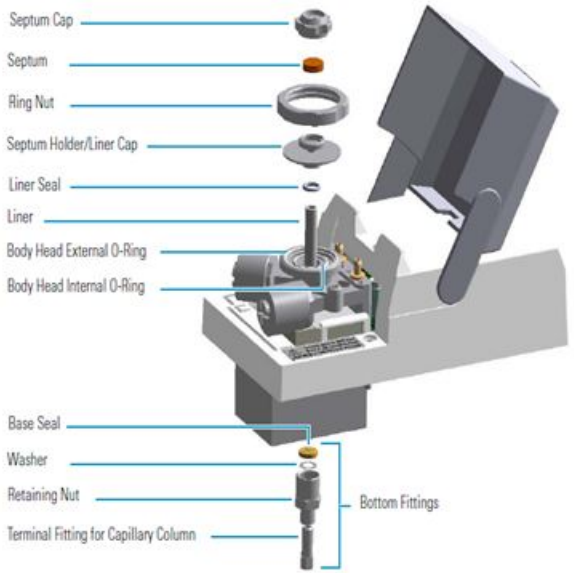
- BTO non stick septum: Up to 400 ° C (Standard septum)
- Blue" thermolite" 340 ° C (Less bleeding)

Septa with "center-guide"

- Merlin septa: No bleeding, the whole is already punched, lasts a long time, but is expensive and needs a special gauge on the needle
- Typical mistake: Overtightening the septum
  After just a few injections there will be a leak in the septum
- Typical maintenance: Every 150 to 200 injections

Maintenance

- Phenomena to consider maintenance
  - LINER active sites will cause
    - ...degradation of analytes
    - ...absorption of the analyte causing bad transfer to the column: Very broad peakshape
  - SEPTUM: Old septa will cause
    - ... leaks
- Typical frequency
  - Varies highly per application!
  - Liner replacement: Approx. 150 injections
  - Septum: Approx. 200 injections
  - In most labs: Both liner and septum are simultanuously replaced



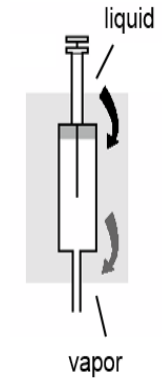
- TRACE 1300 GC benefits for you:
  - The septum is cold, so no burning fingers; liner easily accessable with tweezers
  - SSL module can be completely taken out with 3 screws
  - The SSL body can be removed easily and sonicated
  - For more info: You tube link

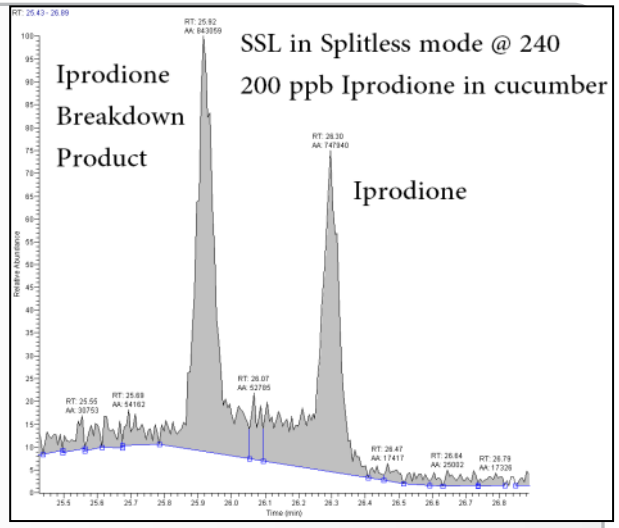
PTV Injection

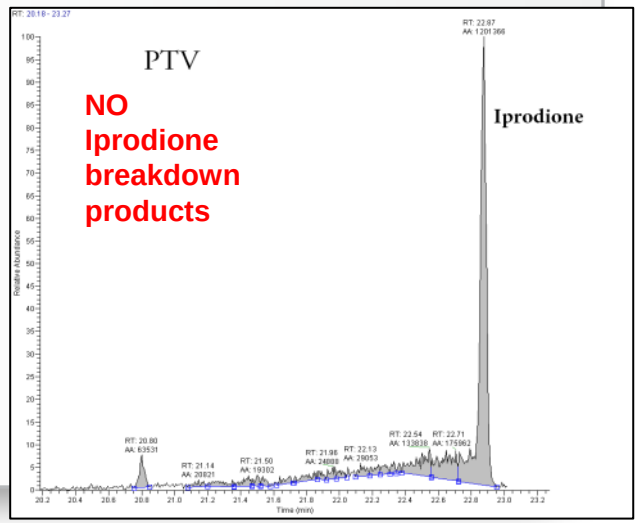
- Important parameters
- Large volume
- Liners and other consumables
- Maintenance

PTV - Key Features

- No needle discrimination
- Less analytes thermal stress
- High transfer efficiency
- Better recovery of less-volatiles
- Low contamination effects
- Splitless and large volume injection mode







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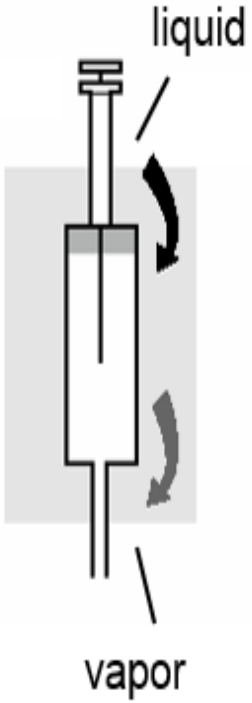
PTV - Splitless Injection Mode – 3 Main Stages

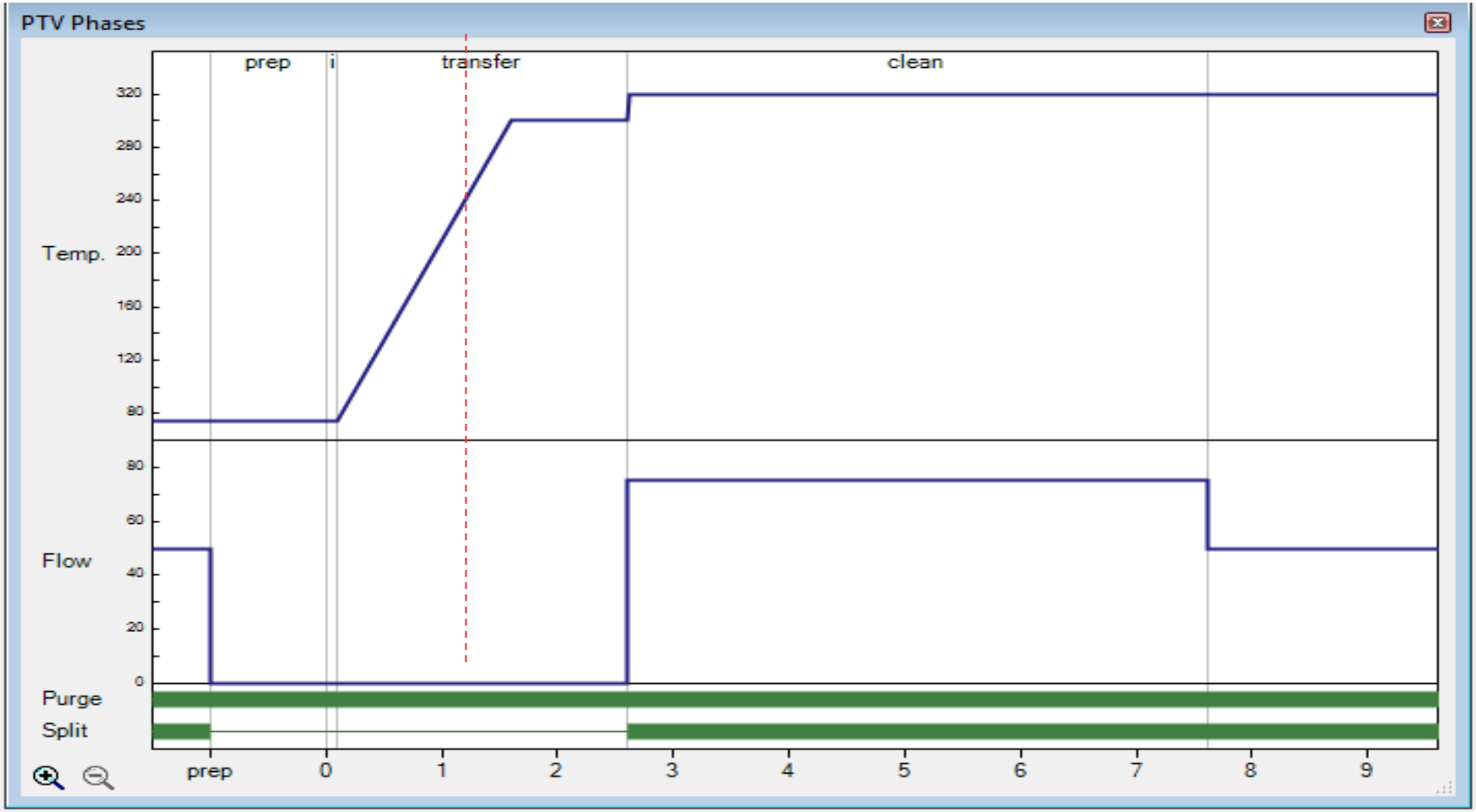


Injection

Transfer

Cleaning

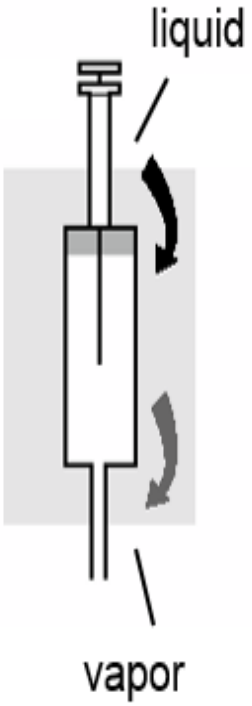
PTV - Splitless Injection Mode - 3 Stages in a Graph



Note split line closure time = Ramp time + splitless time

PTV - Splitless Injection Mode

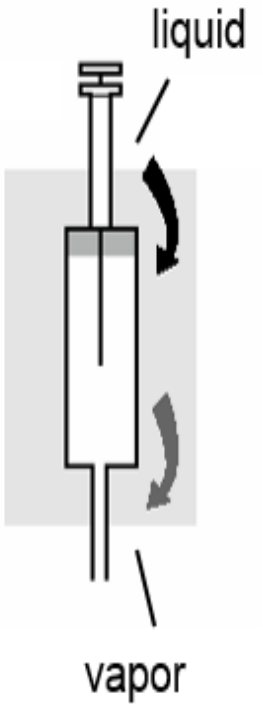
Injection step: Typical parameters



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: Typical parameters
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
Temperature (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; typical 1 minute	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:
Heating rate comparison

Slow heating rates ensure better performances in terms of recovery and repeatability

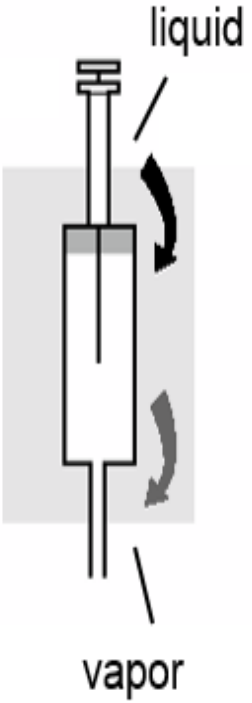
Compound	2,5°C/s		14,5°C/s	
	Average area	%RSD	Average area	%RSD
Lindane	0,79	2,03	0,72	3,72
Aldrin	1,53	1,04	1,47	2,59
Dieldrin	1,58	1,40	1,51	3,82
Eldrin	0,79	3,09	0,64	5,47

Compound	2.5 °C/s		14.5 °C/s	
	Average Area	%RSD	Average Area	%RSD
Diazinon	3766.8	3.1	1967.9	9.2
Isazophos	6652.1	3.2	3724.4	9.1
Chlorpyrifos-methyl	3362.3	2.8	2176.0	10.6
Pirimiphos-methyl	3099.1	4.2	1885.5	8.6
Fenitrothion	2764.0	3.5	1992.7	9.2
Chlorpyrifos-ethyl	3978.1	1.8	2573.3	6.6
Pirimiphos-ethyl	6946.2	3.8	4293.2	8.6
Quinalphos	11848.5	3.2	8708.2	7.7
Pyridaphenthion	4142.7	5.0	3786.3	16.1
Phosmet	13657.2	3.6	12735.6	9.1
EPN	10923.6	4.2	9402.3	12.4
Phosalone	10899.2	3.5	9542.4	6.8
Azinphos-methyl	8556.4	2.7	8542.9	9.7
Pyrazophos	8294.5	3.3	7149.3	11.2
Azinphos-ethyl	11450.3	2.5	9263.7	9.0
Pyraclofos	4624.2	4.0	4746.6	11.5

PTV - Splitless Injection Mode

Cleaning step: Typical parameters

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

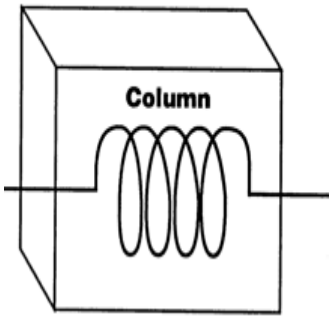


Parameters	Setting	Aim
Ramp (PTV heating rate to clean the liner)	14,5°C/s	Fast heating ramps allow better evaporation of residual matrix
Temperature (Maximum temperature for evaporating residuals)	Matrix dependent	Maximum cleaning temperature must be high enough to evaporate all the matrix in order 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

PTV - Splitless Injection Mode

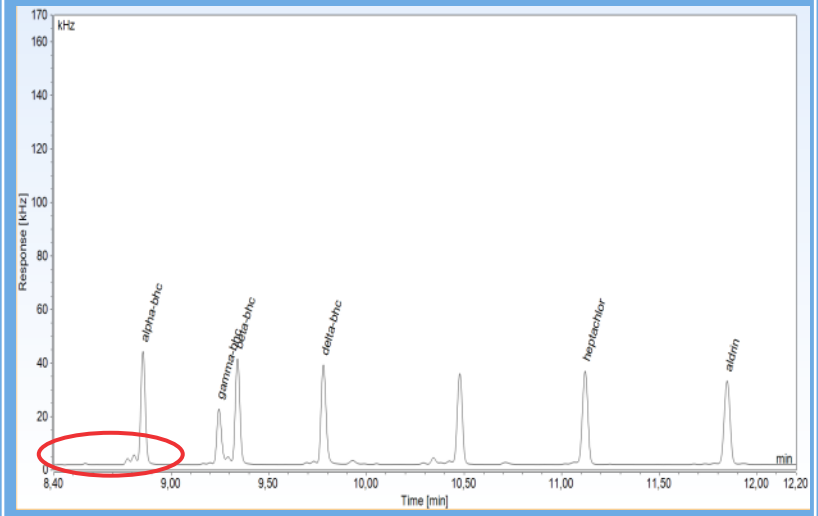
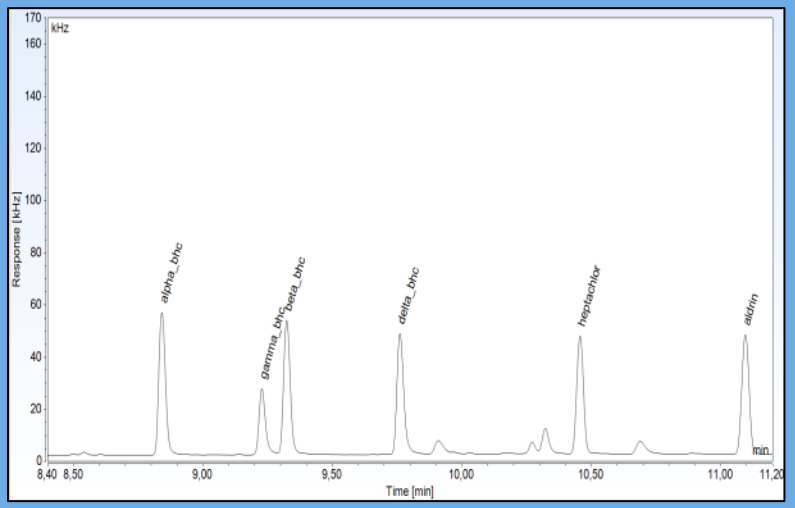
Oven intital temperature typical setting

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 when injecting so much that there is flooding, typically for more polar solvents and for slightly larger volume as 1-2 µl

PTV Injection - Flooding

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

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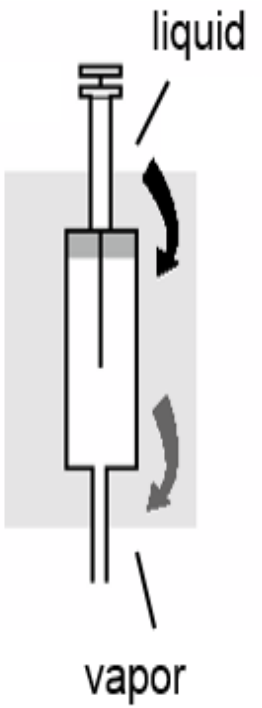
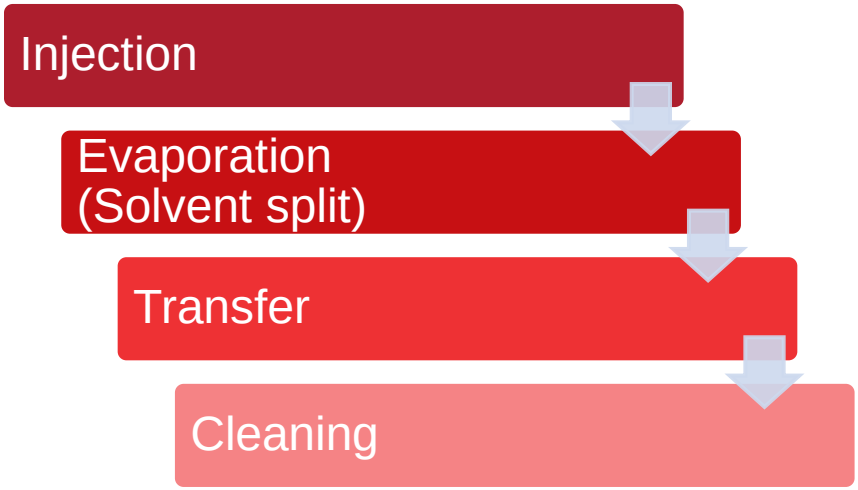
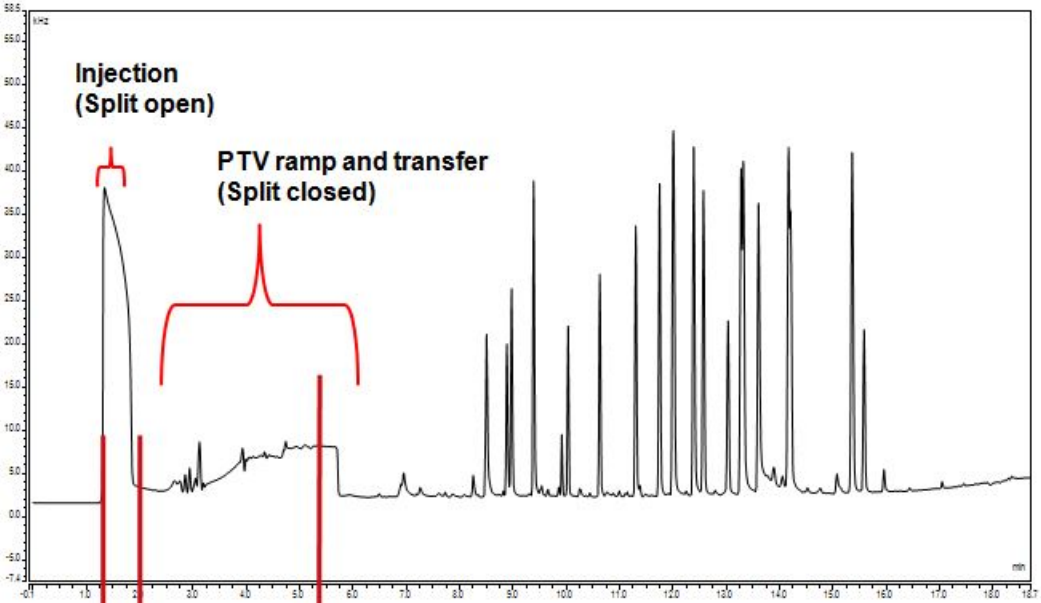
PTV - Large Volume Injection Mode

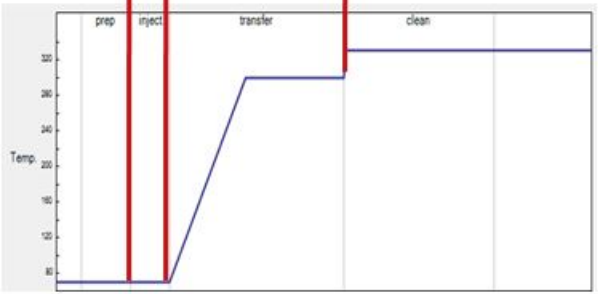


- Higher sensitivity thanks to solvent vent:
  - Up to hundreds µL injection volume
  - Sample pre-concentration during solvent evaporation
- Efficient transfer of low and high boiling compounds
- Trace and ultra-trace analysis
- Reduced sample preparation steps
- Decreased original sample size and lower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



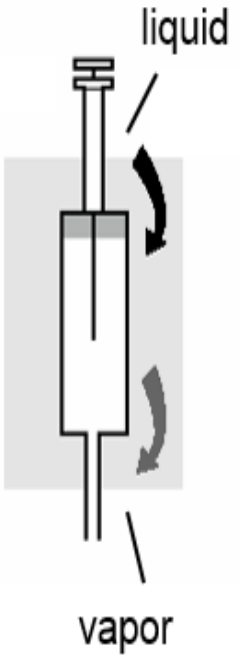
LVI Injection Steps





PTV phases

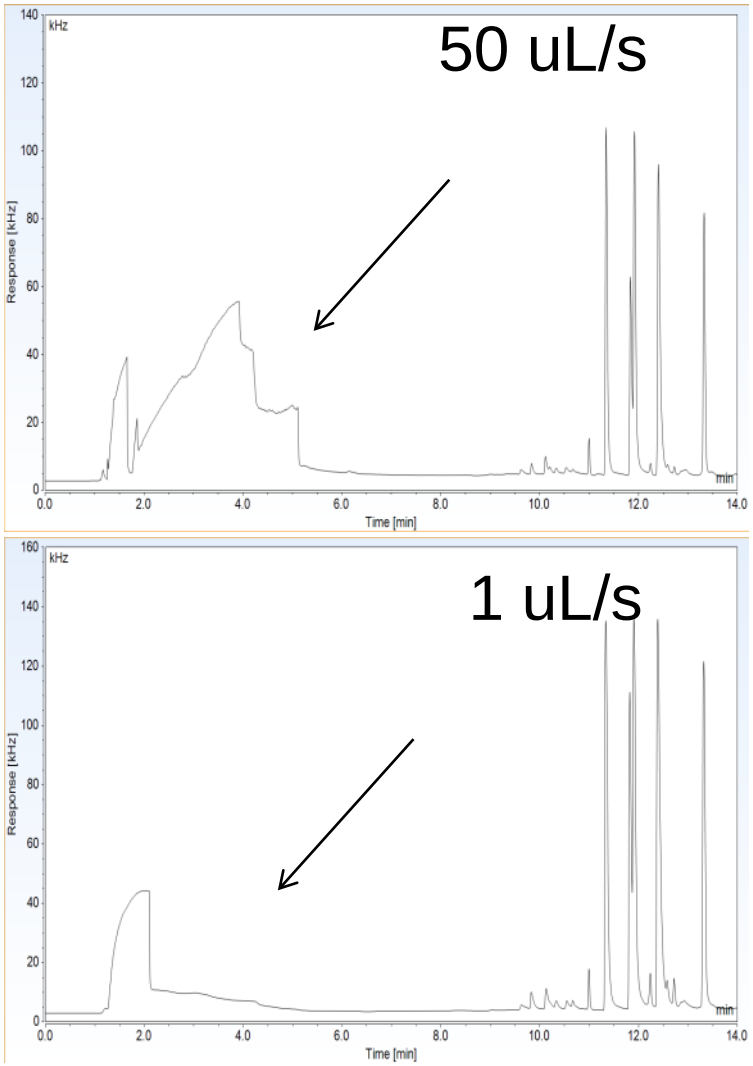
PTV - Large Volume Injection Mode



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 LV - Injection Speed

10 uL injection volume solvent vent at different injection speeds



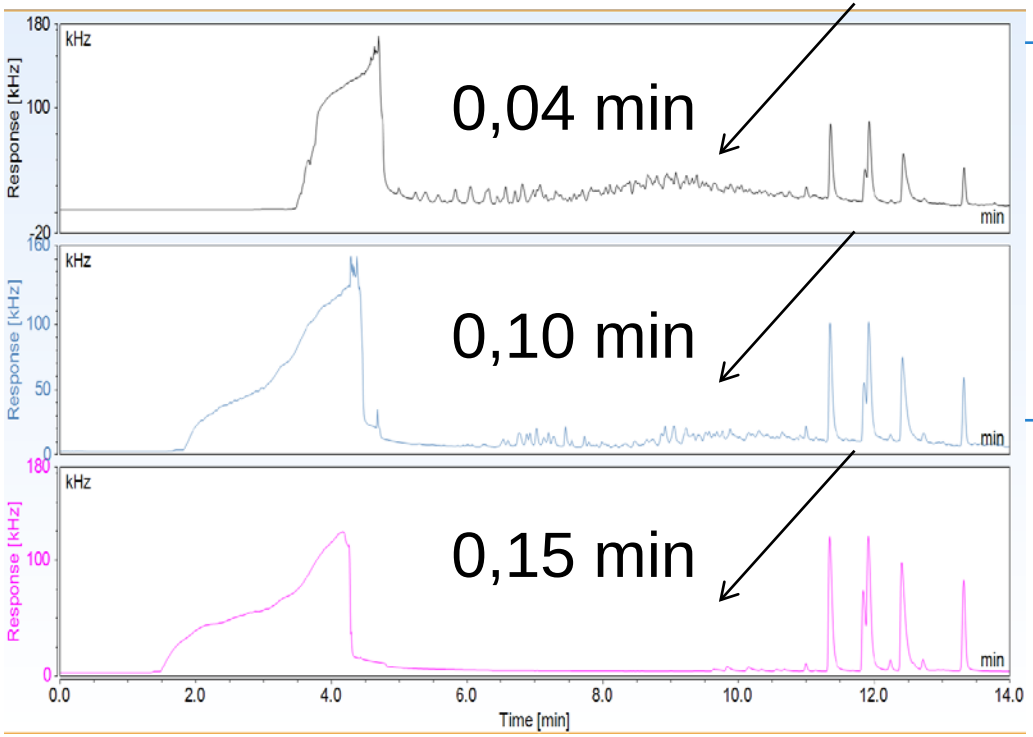
PTV	**75°C hold 0,50 min** **2,5°C/s to 300 °C hold 2 min** **14,5°C/s to 330°C hold 5 min**
SPLIT FLOW	**50mL/min**
SPLITLESS TIME	**2 min**
SEPTUM PURGE	**5 mL/min – CONSTANT**
CARRIER FLOW	**1.2 mL/min**
OVEN	**40°C hold 3 min, 22°C/min to 180 °C, 5°C/min to 270°C, 30°C/min**
ECD	**to 320°C hold 3 min**
LINER	**300°C**
	6-baffles

Slow injection speed (1uL/s) allows a progressive more efficient solvent evaporation during injection step

Injection time must be long enough to complete the injection + solvent vaporization

PTV LV - Injection Time

5 uL injection volume solvent vent at different injection times



PTV	**75°C (see chromatograms for inj time) 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	**1 uL/s**
SPEED	**6-baffles**
LINER	

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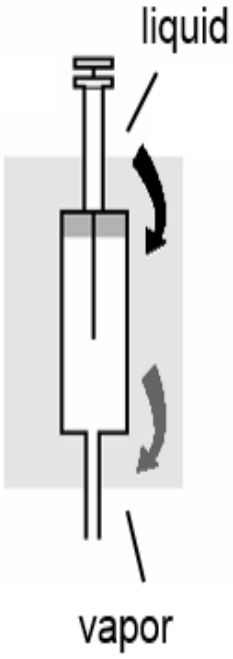
PTV – LV Evaporation Step

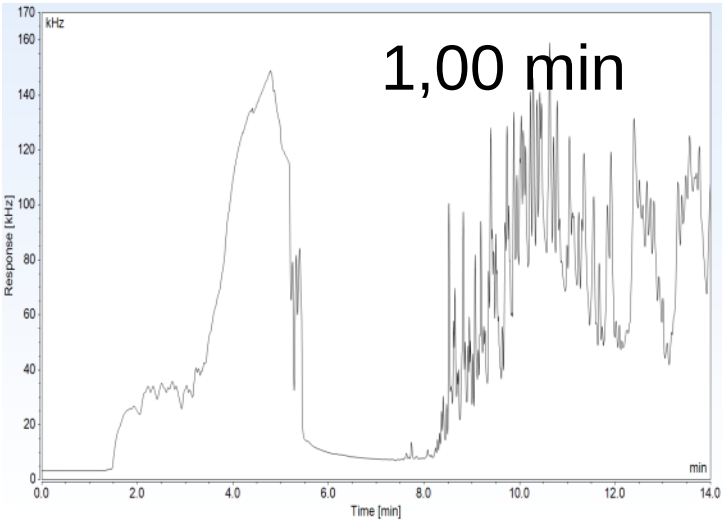


Parameters	Setting	Aim
Ramp (PTV heating rate to vent the solvent)	14,5°C/s	Complete vaporization of solvent and minimized loss of volatile compounds
Temperature (Maximum allowed temperature for solvent venting)	Close to the pressure corrected solvent BP	
Time (Time for solvent)	Solvent volume dependent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

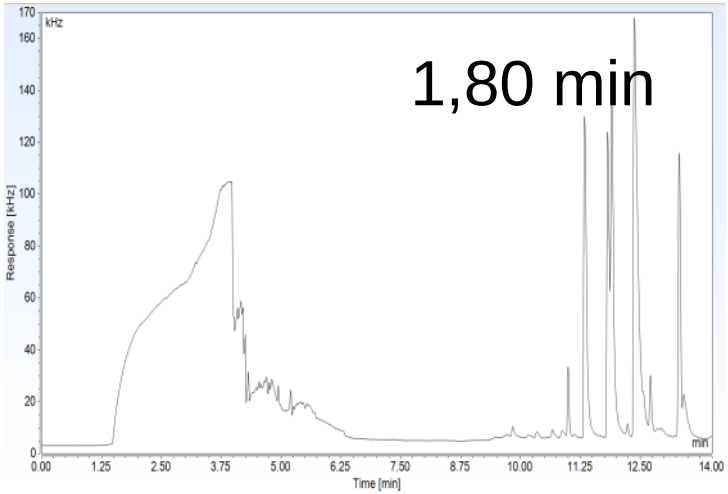
Please note: This is an optional step
Recommended to be added when injecting over 10µl or in case flooding occurs

PTV LV – 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
ECD	320°C hold 3 min
INJECTION	300°C
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	2 min
TIME	5 mL/min – CONSTANT
SEPTUM	1.2 ml/min
PURGE	40°C hold 3 min, 22°C/min to 180 °C, 5°C/min to 270°C, 30°C/min to
CARRIER	320°C hold 3 min
FLOW	300°C
OVEN	1 uL/s
ECD	Sintered
INJECTION	
SPEED	
LINER	

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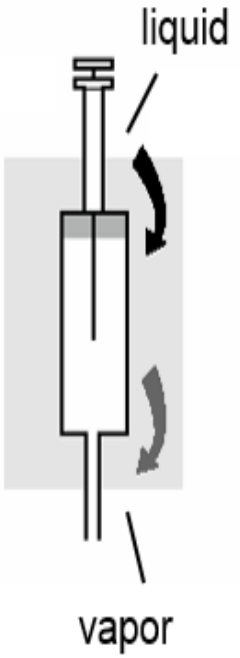
PTV LV - Transfer



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
Temperature (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 - Liners For Splitless Injection Mode

Liners enable:

- Correct sample vaporization
- Analyte transfer to the column

Liners can be:

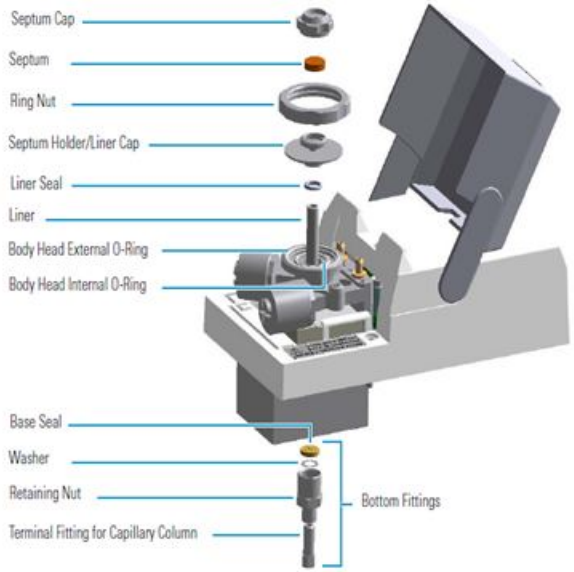
- Straight or baffled
- Empty, packed, sintered
- 1 or 2 mm ID
- Inertness toward the analytes

Need advize for your application?
We are here: Ask us at analyze.eu@thermofisher.com

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

PTV - Maintenance

- Phenomena to consider maintenance
  - LINER active sites will cause:
    - ...Degradation of analytes
    - ..Absorption of the analyte casuing bad transfer to the column: very broad peakshape
  - SEPTUM: Old septa will cause
    - ...Leaks
- Typical frequency
  - Varies highly per application!
  - Liner replacement: approx. 150 injections
  - Septum: approx 200 injections
  - In most labs: both liner and septum are simultanuously replaced



- Thermo Scientific™ TRACE™ 1300 series GC benefits for you:
  - The septum is cold, since the PTV is a "cold"injector; liner easily accessable with tweezers
  - PTV module can be completely taken out with 3 screws
  - For more info: You tube link

Backflush

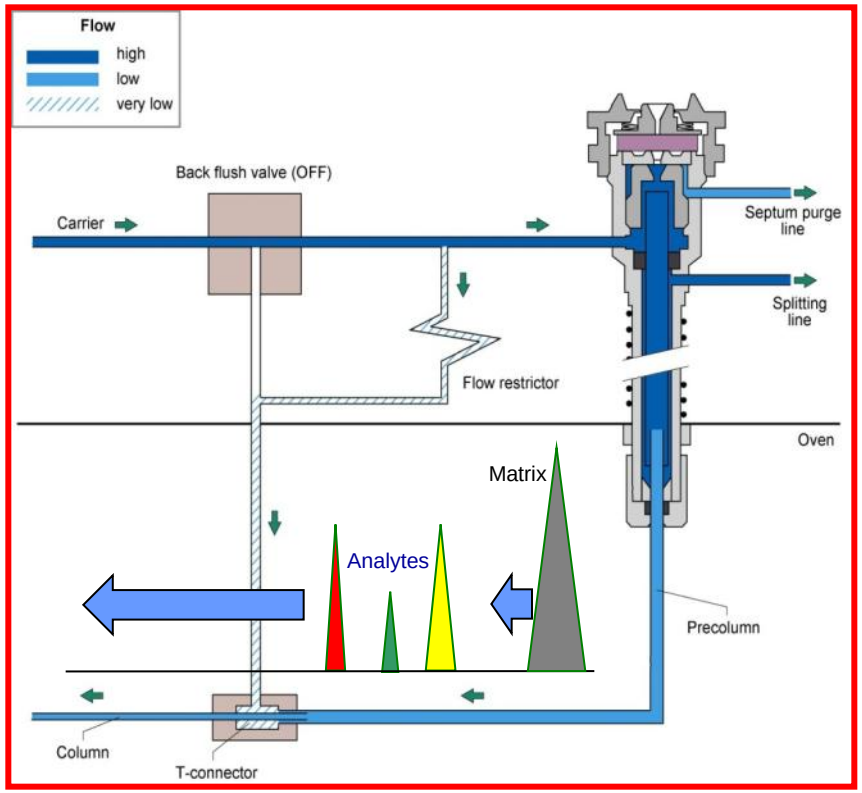
- Important parameters and considerations
- Liners and other consumables
- Maintenance

PTV in Backflush Mode

- Why was backflush developed?
  - Main reason: Column degradation caused by high boiling matrix compounds, such as sugars, sterols, triglycerides,….
  - The phase of the column ends up in a MS source and dirties up the surfaces.
    - Protection of the MS source
    - Longer column lifetime
  - And to possibly shorten the analysis time

PTV Backflush Operation (1)

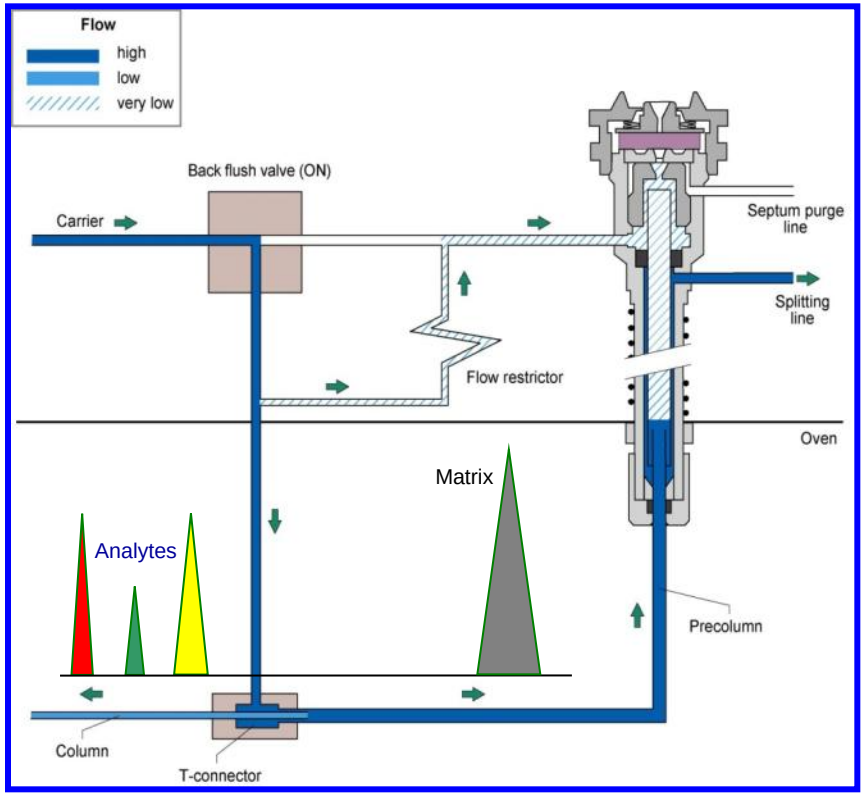
Injection
Transfer to pre-column and analytical column



- Analytical set up
  - 3-way valve in carrier gas line
  - 2 m pre-column
    - 0.53 mm ID, deactivated
  - T- connector to column
  - Restrictor
    - To purge the T-piece, and flush the inlet during backflush
- During injection
  - Standard carrier gas flow
    - Compounds move through pre-column
  - High boilers travel slowly

PTV Backflush Operation (2)

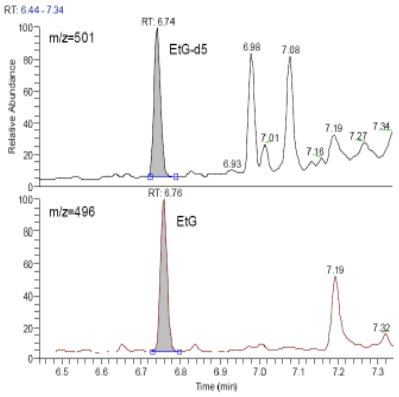
Backflush
Clean pre-column and Injector during analysis run



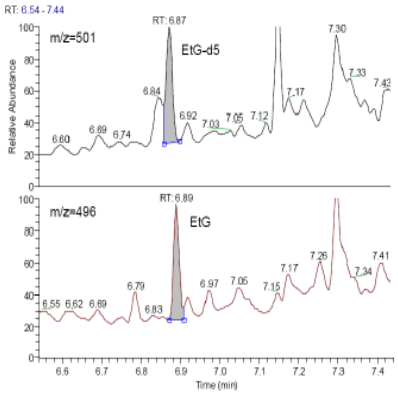
- Activate backflush
  - 3-way valve switches
    - After last compound of interest entered the analytical column
  - Carrier flow is redirected
    - Analysis runs as usual
    - Pre-column is back-flushed
- Backflush operation
  - On during analytical run (Concurrent to analysis)
  - High boilers get backflushed
    - Through regular split outled
  - Insert liner gets cleaned
    - By restrictor line

EtG with GCMS and PTV Backflush

SSL Injector

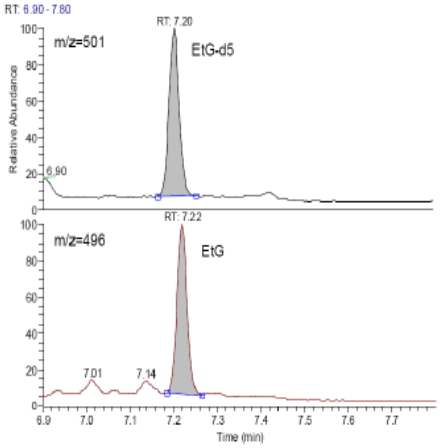


after maintenance work

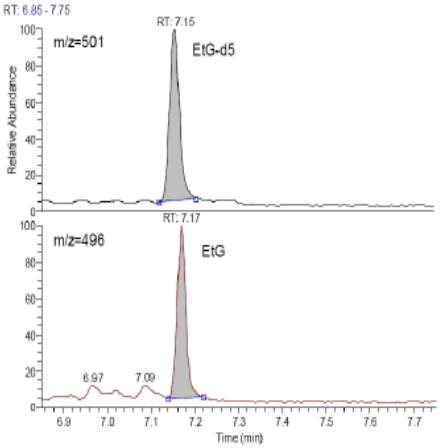


after 30 injections

PTV Injector with Back-flush



after maintenance work



after 30 injections

Data courtesy from BRECHBÜHLER

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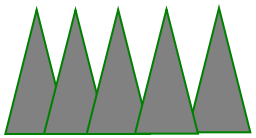
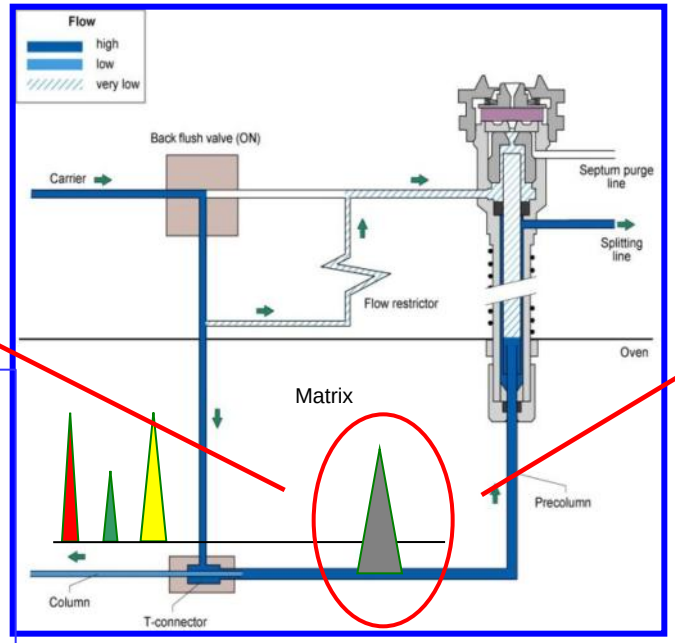
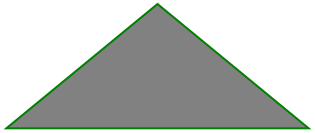
PTV Backflush – Pre-Column Choice







- **Coated column with phase**
- More precise separation of matrix and target analytes
- 1 m*0.53 mm ID
- Usually 0.1 µm film thickness will do!
- Pro's: Better separation
- Con's: Price, and backflush time starts later

- **Uncoated, deactivated precolumn**
- Very course separation of matrix and target analytes
- 2 m*0.53 mm ID
- Pro's: Cheap and easy
- Con's: Sometimes separation is needed

Common Issues and Solutions

- Absorption effects
- Peak shape degradation

Matrix Effects

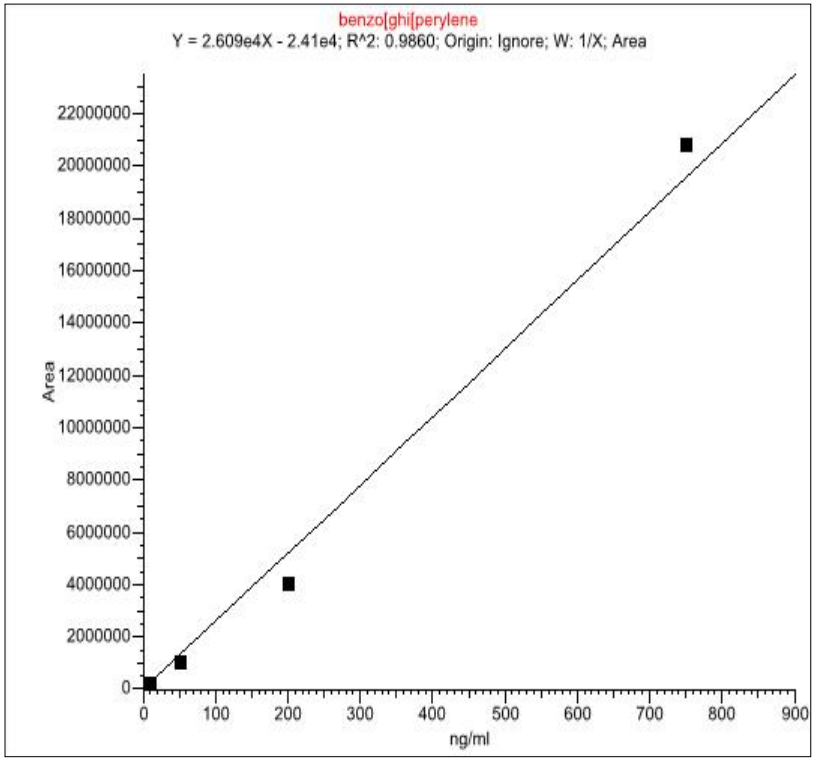
- Effect of the sample matrix (By-products) on the chromatography of the solutes of interest:

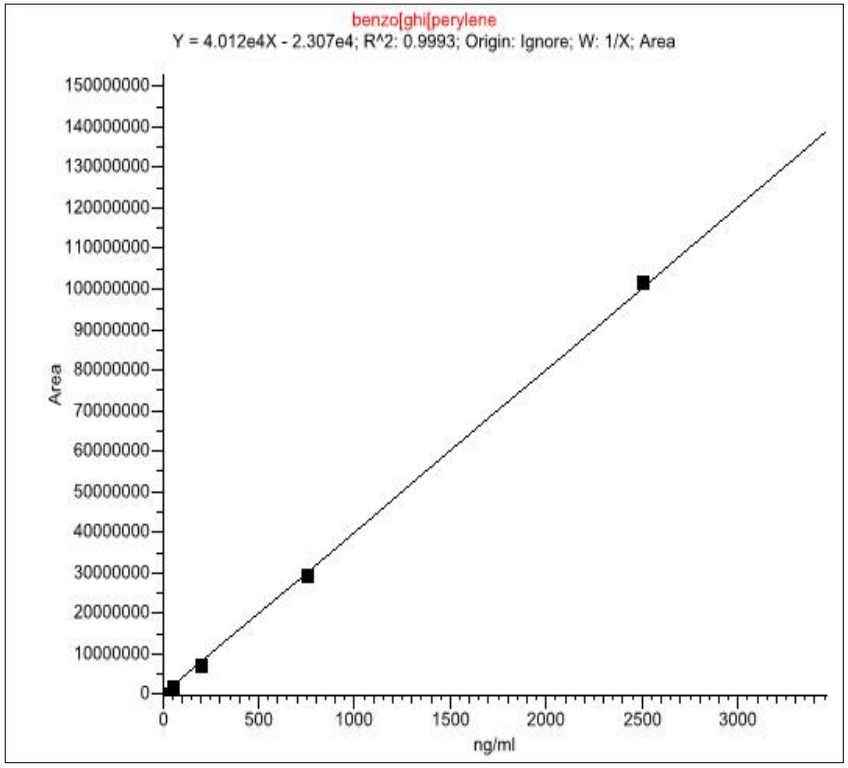
  Calibration with clean solution may provide wrong results.

- Solution: Calibrate in the matrix
- Solution: Use internal standards
- Solution: Standard addition method

Compound	BrandA	BrandB
Methamidophos	1.51	1.28
Acephate	1.83	1.36
Omethoate	1.78	1.23
Dimethoate	1.44	1.11
Diazinon	1.27	1.06
Ethion	1.36	1.09
Parathion methyl	0.9	

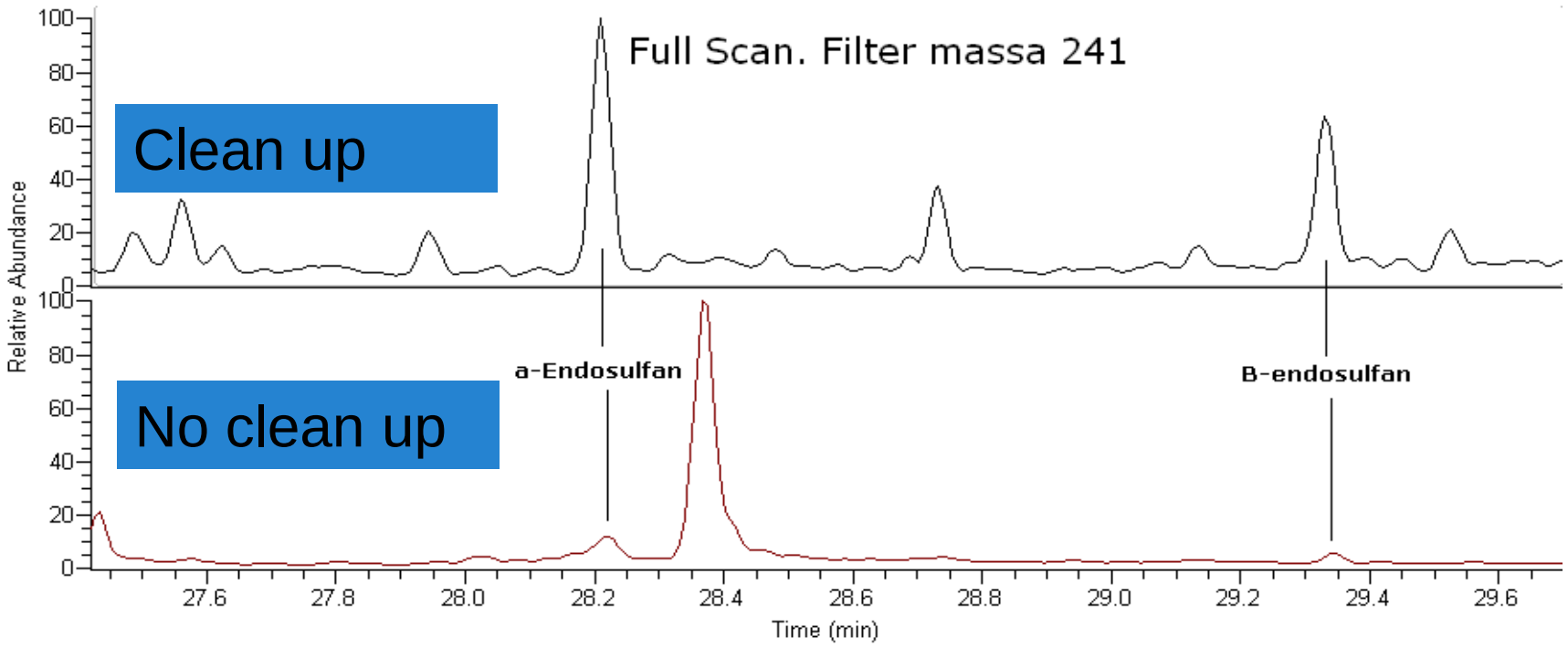
Linearity Issues – Liner Needs Matrix





- Typically: All liners have active sites that need to be disabled. Matrix does that job perfectly well.
- In case of adsorption issues inject approx. 5 times with matrix after liner replacement or use analyte protectants to cover the active sites.

Why Do We Perform Sample Clean Up? Chromatography



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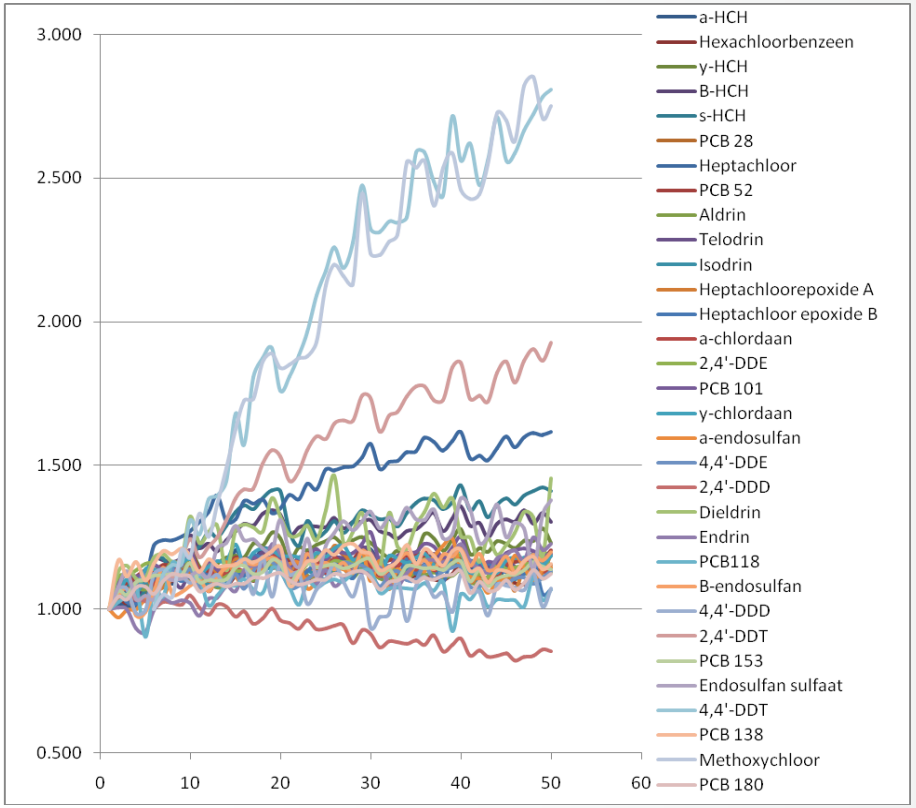
Adsorption in the Liner

- Chlorinated pesticides in sediment sample
  (= Uncleaned, heaviest environmental matrix)

- 50 injections:
  - Breakdown of DDT
  - Increase of DDD
  - Increase of methoxychloor

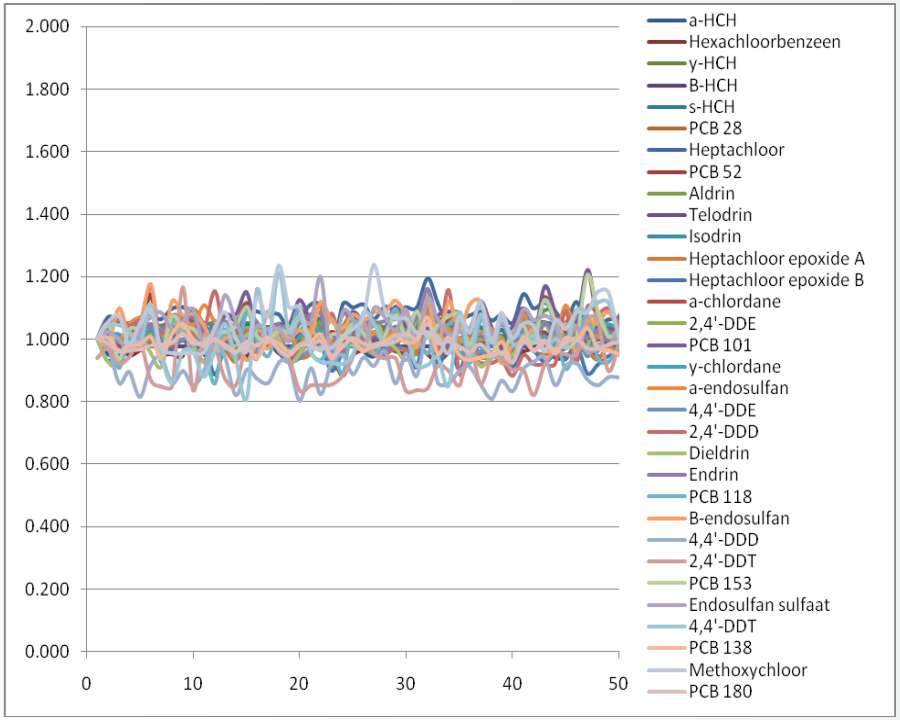


Sample Clean Up - Robustness

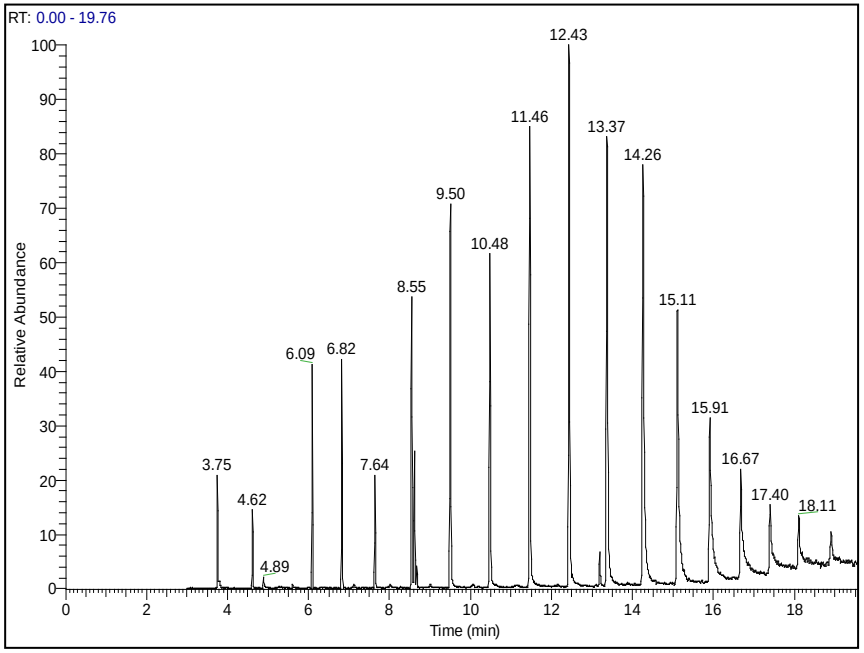
- Chlorinated pesticides in sediment sample, with clean up

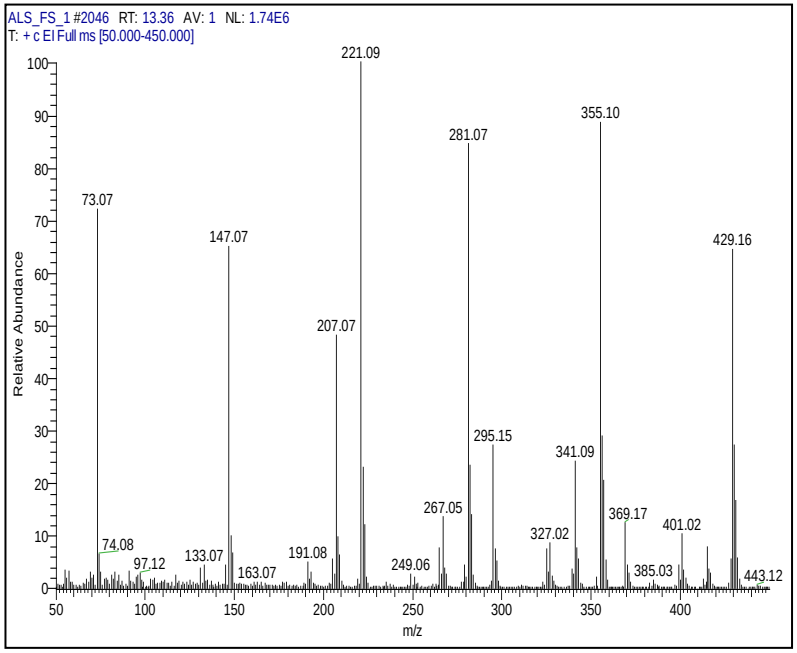
  (Cleaned, heaviest environmental matrix)

- 50 injections



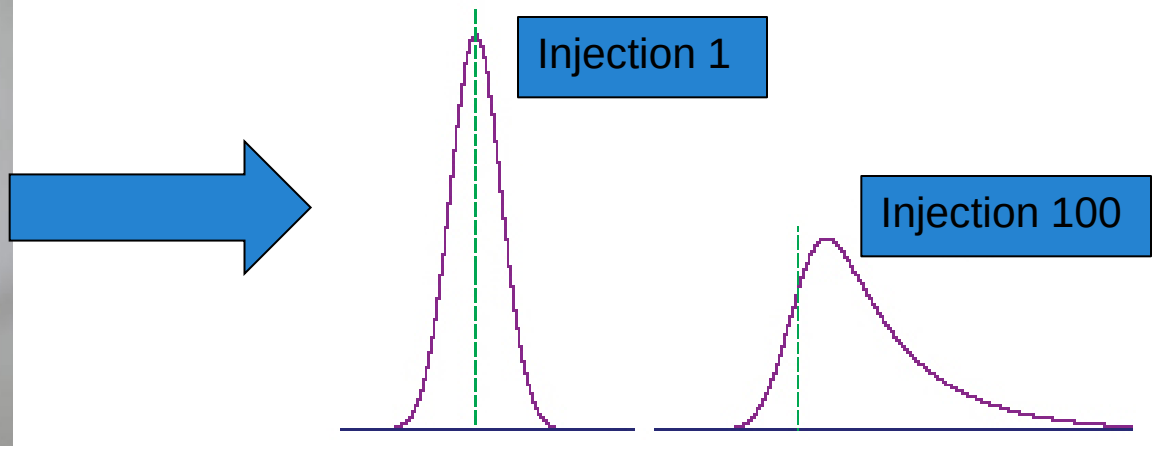
Septum Material - Inside the Vial





- Typical chromatogram and spectrum of septum bleed
  - The source is almost always the septum of the vials, especially when stored for a longer time or when pierced several times.
  - But another source is the septum of the injector port itself: It will have the same pattern!

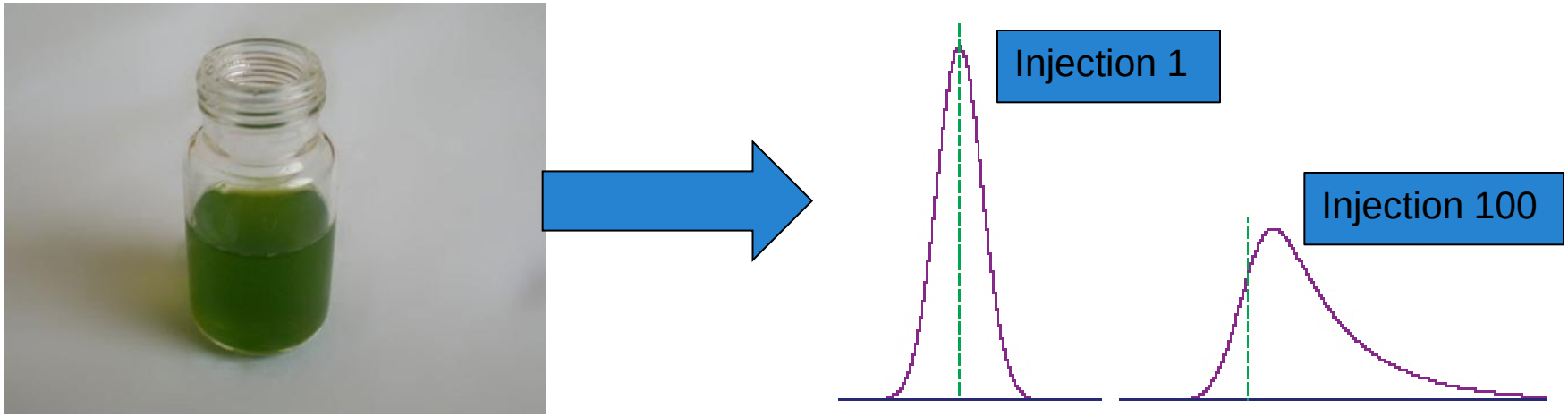
Column Quality - Protecting the Column (1)



- Some samples contain a lot of particles.
- The particles will have an effect on the column and eventually the peaks will tail.

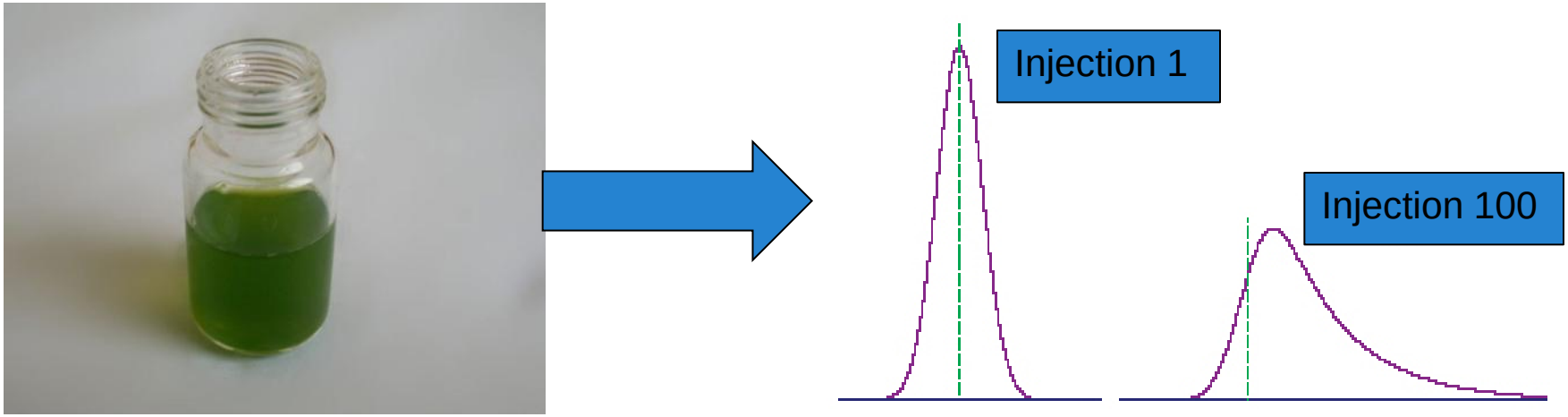
- It is better to filter the extracts.
- Or if this is not possible: Choose a liner with glasswool, it will act as a filter for the column

Column Quality - Protecting the Column (2)



- Some samples contain a lot of heavy matrix like sugars, fats.
- Simply removing 30cm of the front of the column should generate better peakshapes.
- The better solution is to add a guard column in front of the analytical column.
  - Better refocussing (Better chromatography)
  - NO retention time shift when doing maintenance

Column Quality - Protecting the Column (3)

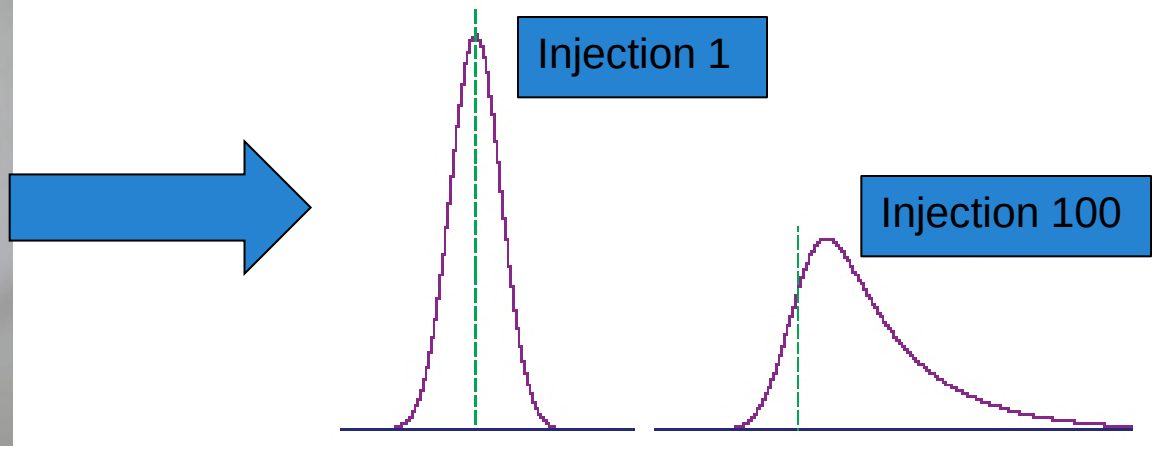


- Some samples contain a lot of heavy matrix like sugars, fats.
- If a guard column does not help and the complete column needs to be replaced in under 500 injections…

  …consider using backflush.

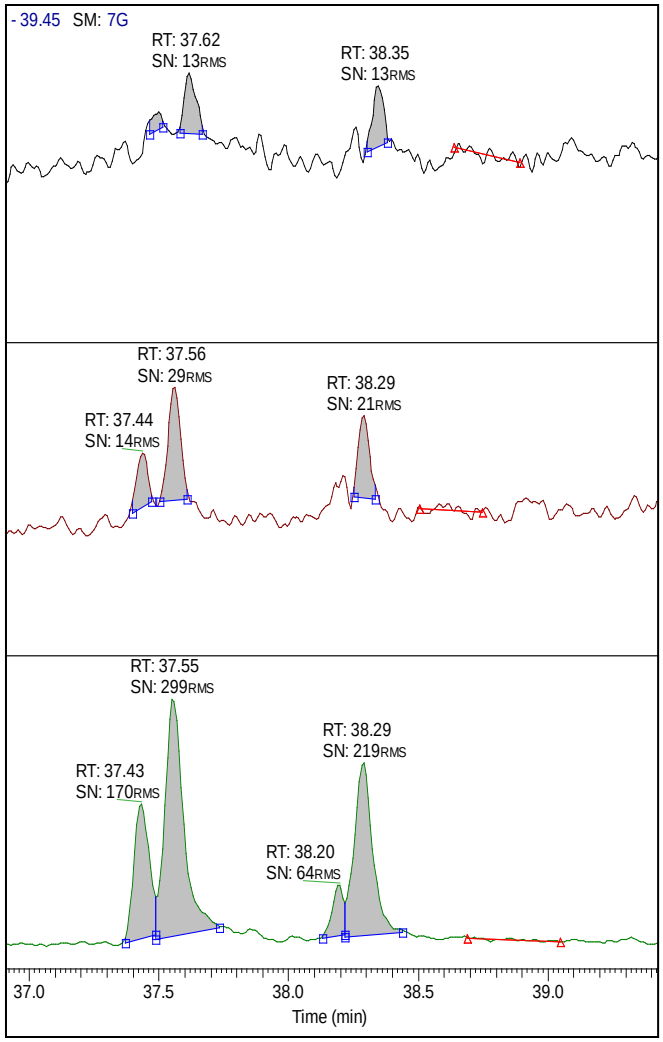
  But take care: If this is a possible solution then it is best to use concurrent backflush so the matrix is eliminated from a pre-column, and never reaches the analytical column.

Take Care! It Can also be the Liner



- In all the previous slides the column got the blame for this phenomenon.
- But adsorption effects in the liner can cause bad transfer to the GC column and so also peak tailing.

GC End Temperature



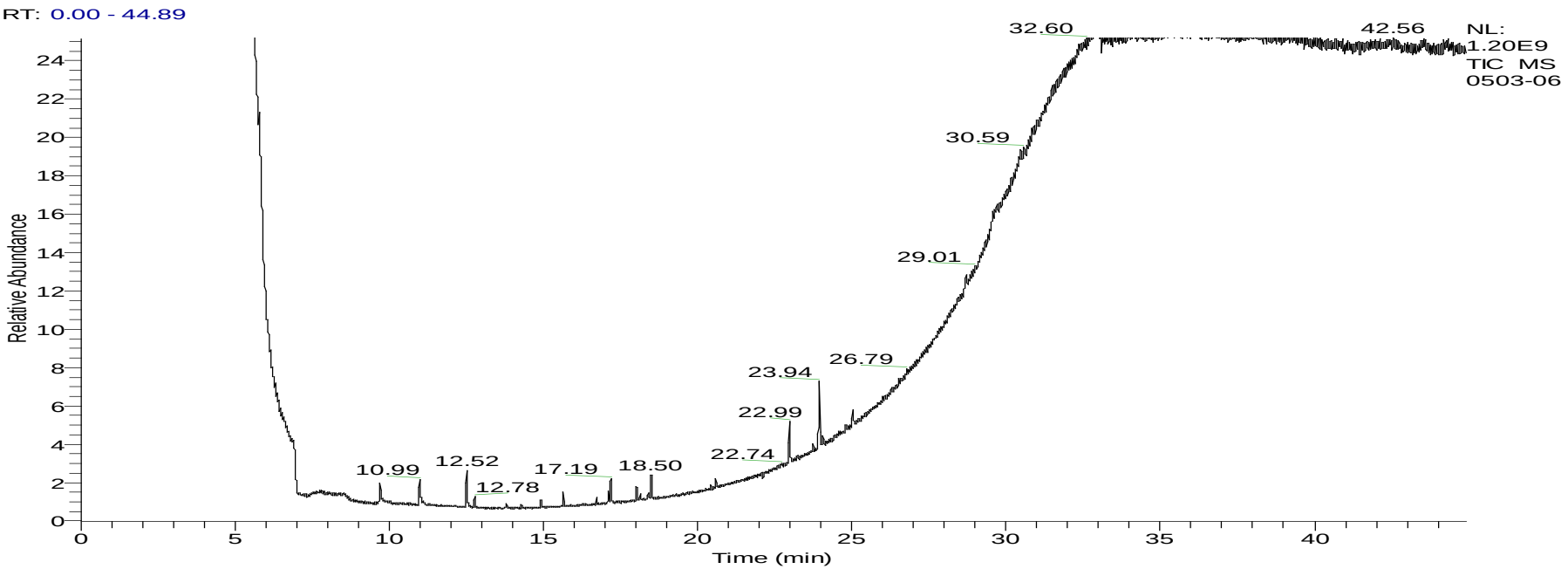
Oven: 40ºC (8min)-10ºC/min-280ºC (01min)-2ºC/min-300ºC (1min)

Oven: 40ºC (8min)-10ºC/min-280ºC (01min)-2ºC/min-300ºC (8min)

Oven: 40ºC (8min)-10ºC/min-280ºC (01min)-2ºC/min-320ºC (8min)

- PAH standards injected after a very heavy environmental matrix
- Make sure the column is clean and all heavy compounds are eluted
- Also if the ramp is fast: The high boilers “move” into the column bleed.

Column Bleed



- Chromatogram in FullScan mode is showing excessive column bleed.
- Normal column bleed has intensities below 1e7.

- Column bleed will end up in the MS source and dirty it up quickly.
- It is not visible in SIM or in MS/MS, so often this is a “hidden” problem.

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- Switzerland: Brechbühler
- Europen GC and GC-MS sales support expert: Elena Ciceri
- Global product specialists: Cristian Cojocariu, Dominic Roberts, Richard Law, Giulia Riccardino
- **Typical profile for every specialist: More then 10 years of GC and GC-MS analytical expertise, in many cases longer!**
- **OR……email: analyze.eu@thermofisher.com**

Resources

- Liner Selection guide
- Chrom Expert site

- Downloadable applications

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Any Questions?

Do you have additional questions or do you want to talk to an expert from Thermo Fisher Scientific?

Please send an E-Mail to **analyze.eu@thermofisher.com** and we will get back to you.