7700 ICP-MS

8800 ICP-QQQ

5977 SQ

7010 QQQ

7200B QTOF

6120 SQ

IR-4 LC/MS TIPS AND TRICKS

Presenter: Stephan Baumann

Agilent Technologies, Inc.; Santa Clara, CA

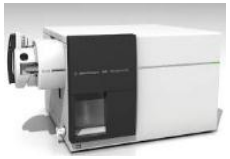
6560 IM QTOF

6130 SQ

6150 SQ

6420 QQQ

6470 QQQ

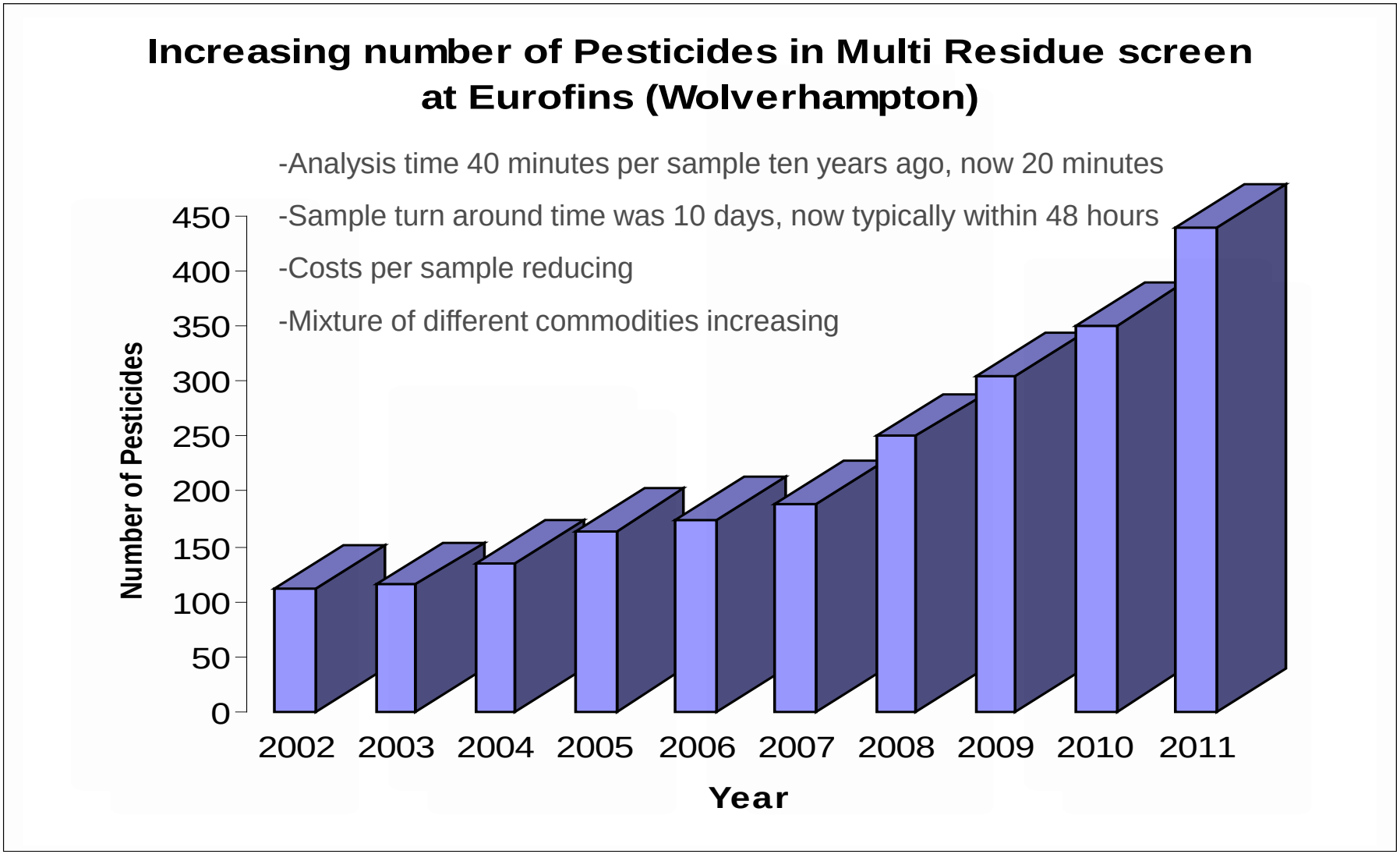


6495B QQQ

6545 QTOF

6550 QTOF

Greater Scope than ever for Pesticide Screening



Regulated Food Safety Surveillance is changing.

- More global supply chains means that Governments and producers are looking to increase the scope of potential contaminants in surveillance schemes especially with respect to pesticides and vet drugs.

How many compounds is it possible to detect?
Progress over last 10 years

LCTQ

GCTQ

In fact it's QQQ's that made all the progress.

LCQTOF

GCTOF

QTOF's have always been able to detect many peaks

Number of compounds	500	1000	1500	∞

450 Pesticides with 1290/6495

See Link to Covance Webinar

Creative chromatography improves the analysis of the early eluting pesticides

Triggered Product Ion scanning cannot be used because quantitation is compromised and scan sensitivity is insufficient. tMRM maintains spectra with MRM sensitivity.

Quant screening seldom reaches 450 compounds by LC/MS?

Covance has deployed parallel methods in USA, Europe & Singapore.

SeparationScience
PREMIER LEARNING FOR ANALYTICAL CHEMISTS

Agilent Technologies

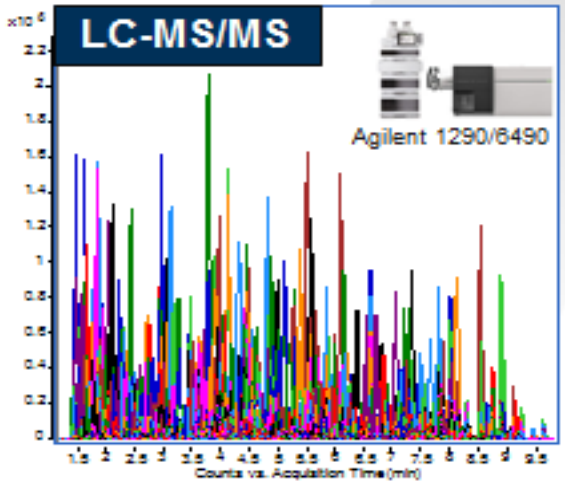
COVANCE
SOLUTIONS MADE REAL

Improved LC-MS/MS Pesticide Multiresidue Analysis using Triggered MRM and On-Line Dilution

Presenter: Dr Katerina Mastovska (Covance Laboratories, Greenfield, Indiana, USA)

LC-MS/MS Analysis – Main Topics

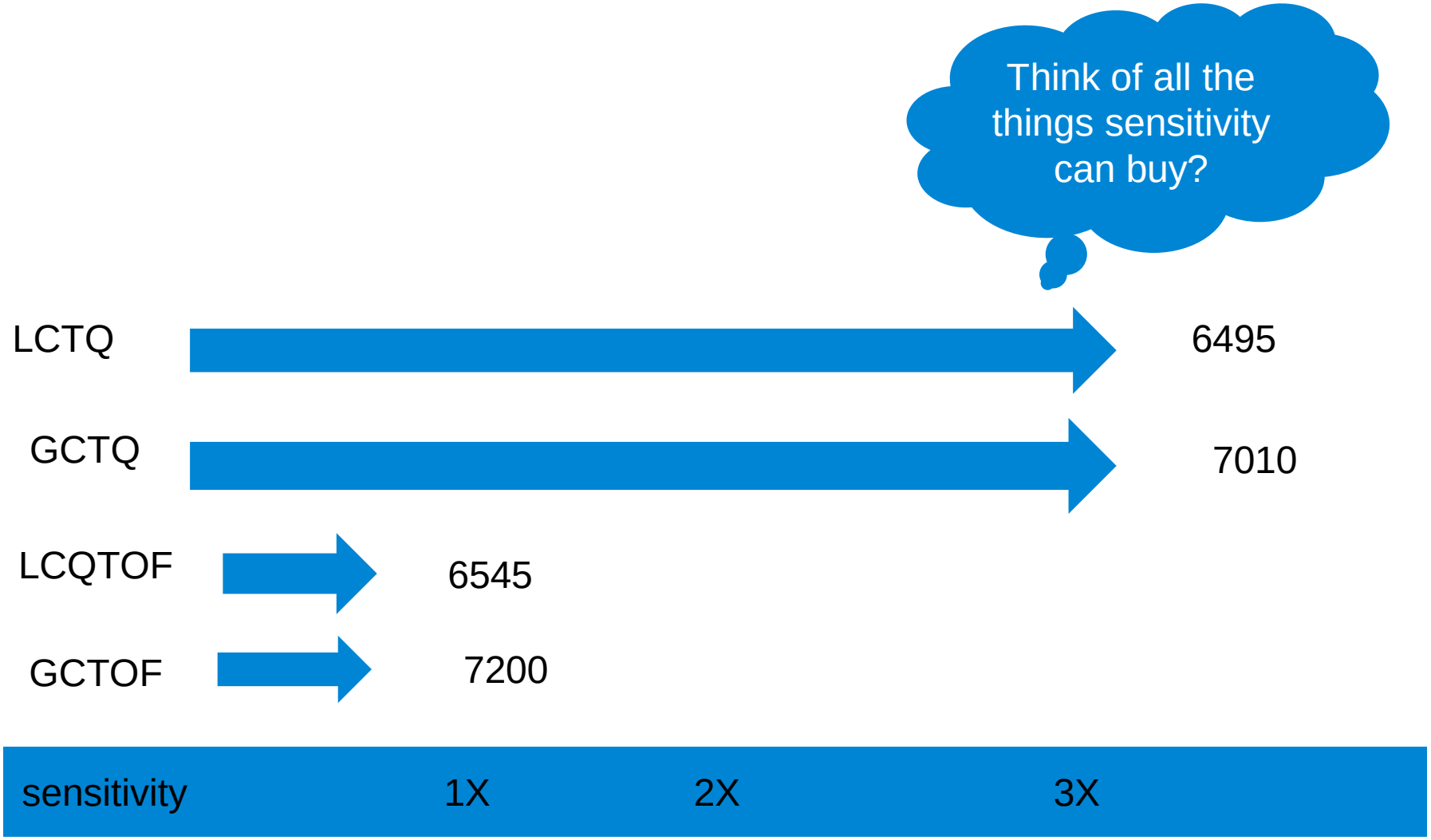
- **On-line dilution**
  Improved chromatography of more polar analytes
- **Triggered MRM**
  Improved identification
- **Development/optimization**
  MS/MS and LC conditions
  for ≈ 450 analytes
  eluting in < 10 min
- **Interlaboratory transfer**
- **Method validation**



9 | Separation Science Webinar, February 16, 2016

COVANCE
SOLUTIONS MADE REAL

Sensitivity : Progress over last 10 years



A Different Approach with QTOF

Q-TOF is an emerging technique which performs qualitative screening as well as quantitative screening.

Allows labs to…..

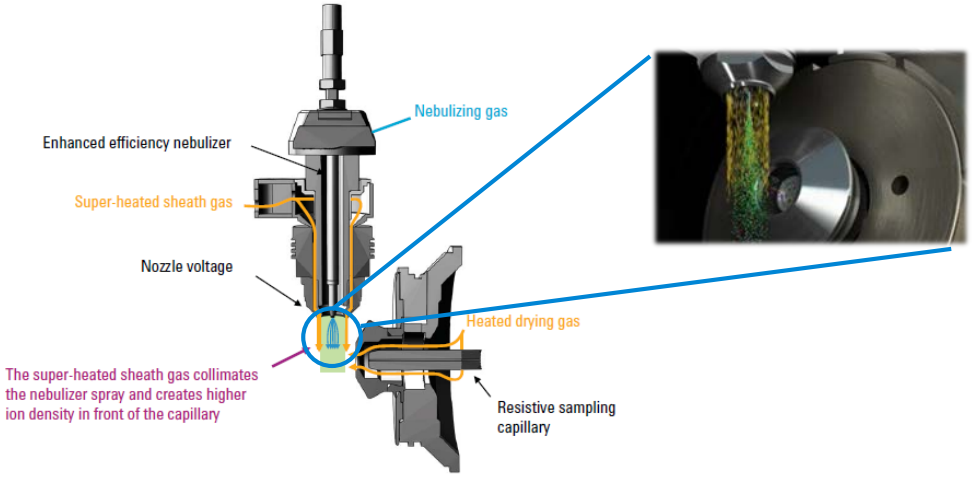
- To reliably detect and identify compounds even without a standard.
- Possibility to also look for new compounds (labs can increase scope)
- Retrospective data analysis.
- Possible to implement quantitative screening too for some compounds.
  - mix & match for different compounds depending on how likely they are to show up.

Why QTOF for regulated surveillance?

QQQ?	✓	✓	✗
QTOF with PCDL library can add certainty to identifications.		✓	✓
PCDL with All ions workflow can verify results even when scope is large.			✓

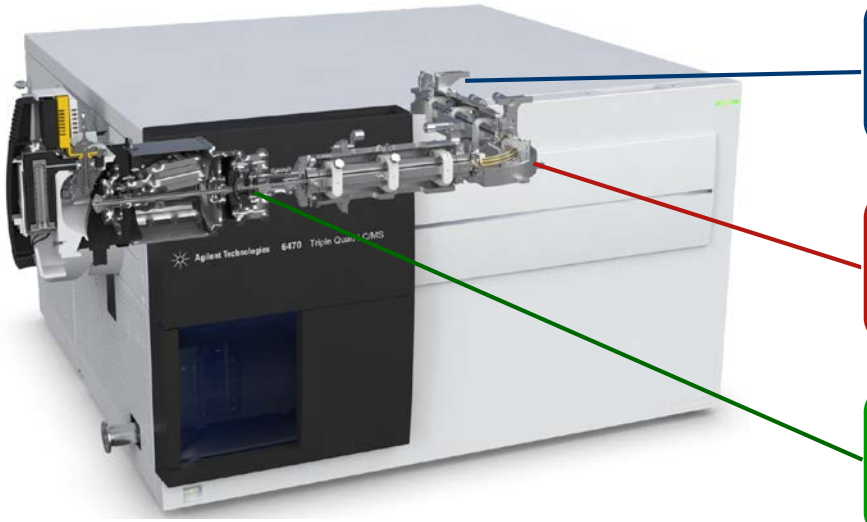
6470 QQQ Technologies

Enhanced Performance in a Smaller Space



Agilent Jet Stream Technology

- Thermal gradient focusing
- Efficient desolvation
- Creates an ion rich zone
- Up to 10x gains in sensitivity



3

- An Ion Detector with High Energy Conversion Dynode and Low Noise
- Improved ion detection

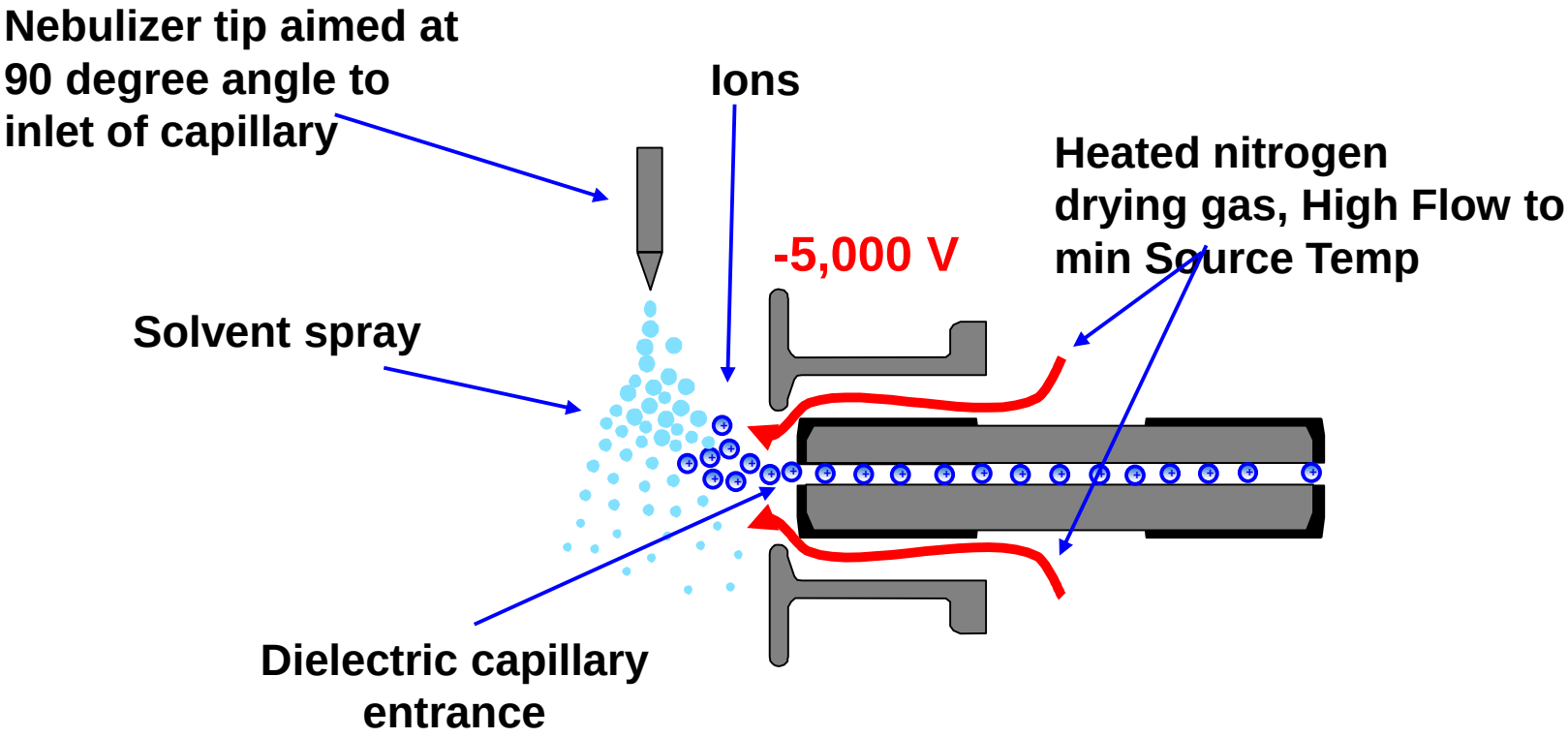
2

- A Curved and Tapered Hexapole Collision Cell
- Effective ion collection

1

- Enhanced Q1 Ion Optics
- Improved ion transmission

Orthogonal Introduction and Electrospray Ionization

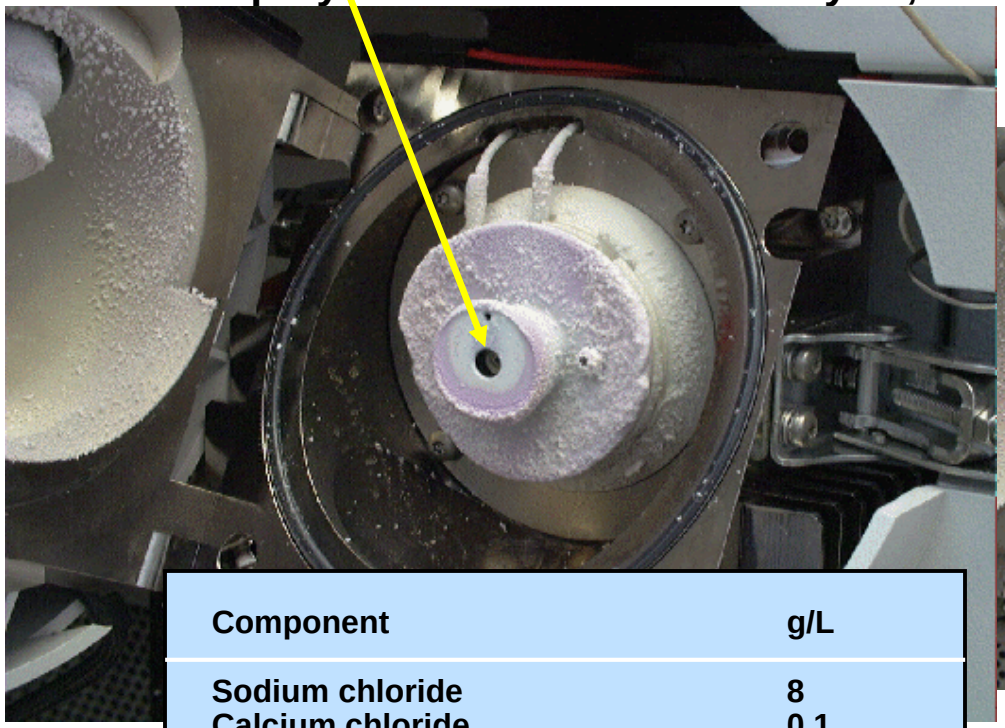


Patented Single Bend 90 degree orthogonal interface

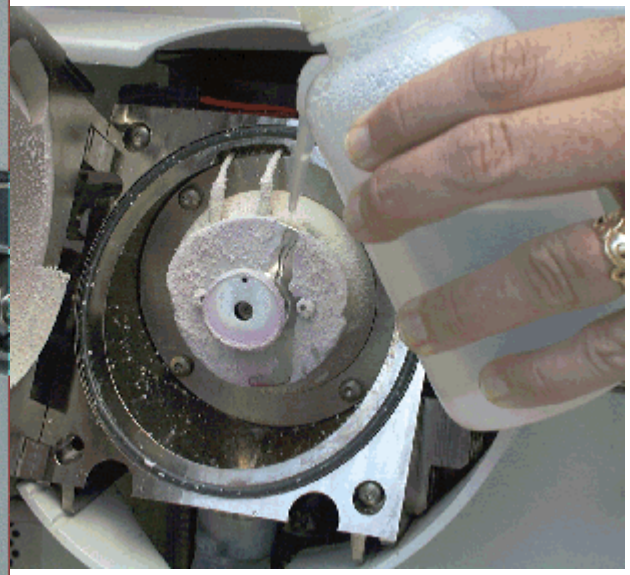
Matrix Tolerance due to Orthogonal Design

APCI Spray Chamber after 635 Injections of Hank's Salt Solution

Flow to spray chamber for entire analysis, entrance to capillary is



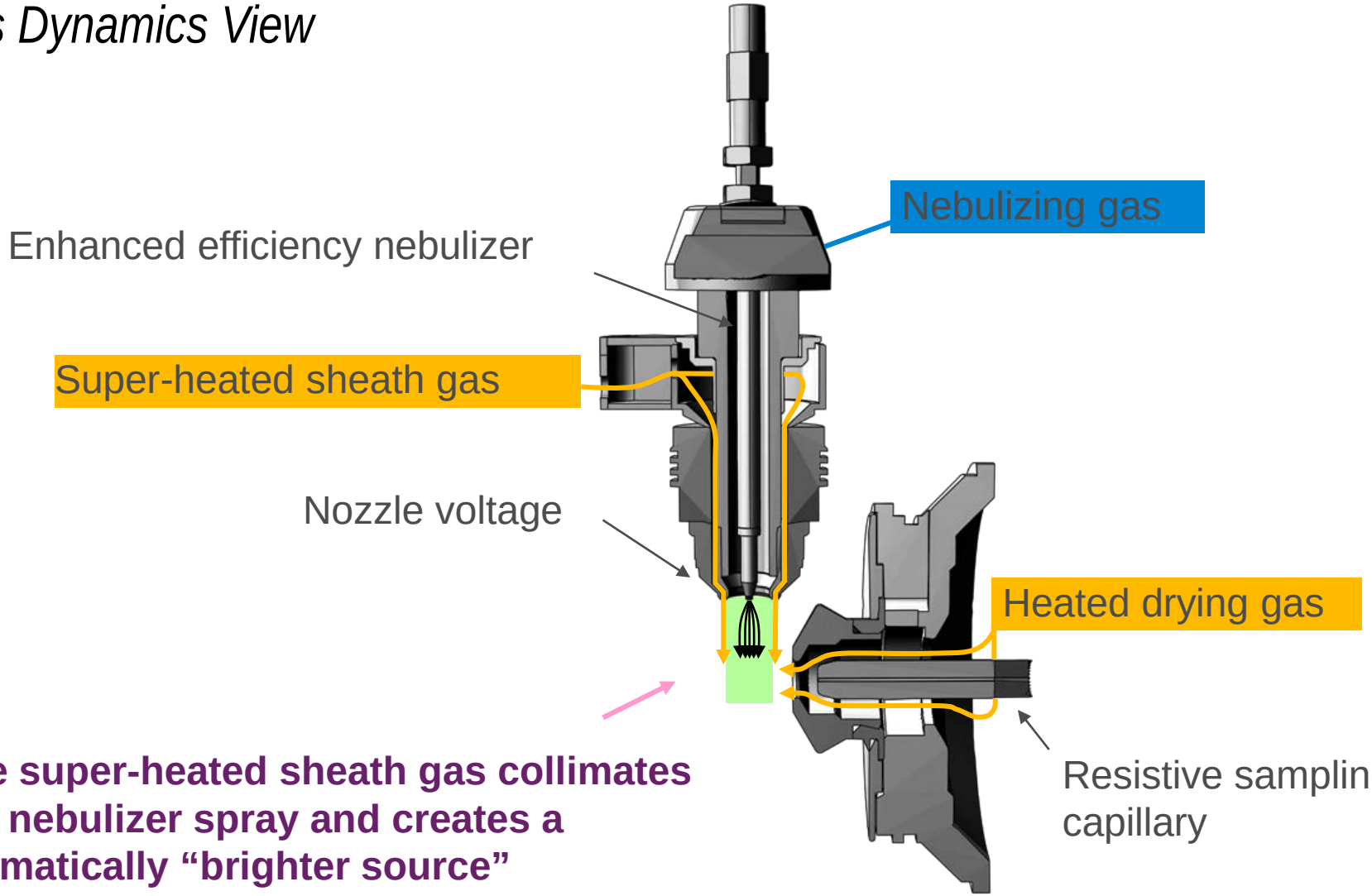
Cleaning the spray chamber



Component	g/L
Sodium chloride	8
Calcium chloride	0.1
Potassium chloride	0.4
Potassium phosphate monobasic	0.06
Magnesium Sulfate	0.1
Sodium bicarbonate	0.35
Sodium phosphate dibasic	0.048
Glucose	1
Phenol red	0.011

Agilent Jet Stream Ion Generation

Gas Dynamics View

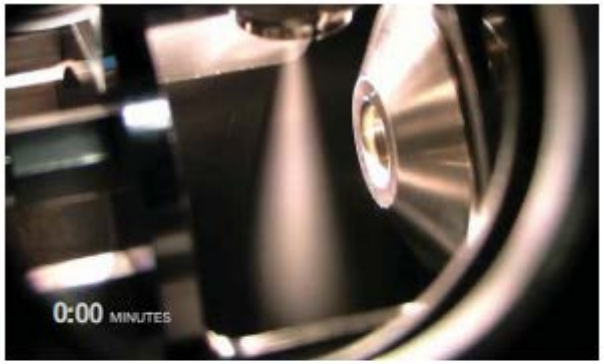


The super-heated sheath gas collimates the nebulizer spray and creates a dramatically "brighter source"

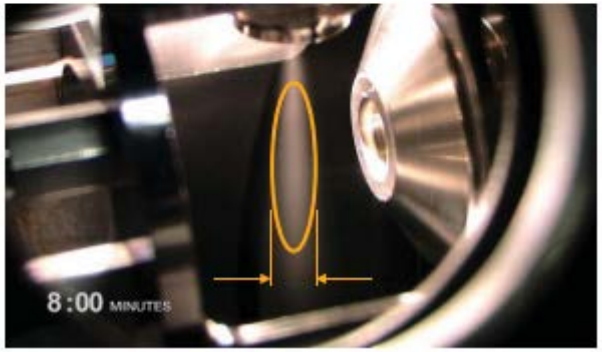
Agilent Jet Stream Ion Generation

Adds 3 to 10 x detection by focusing the Taylor Cone

Off

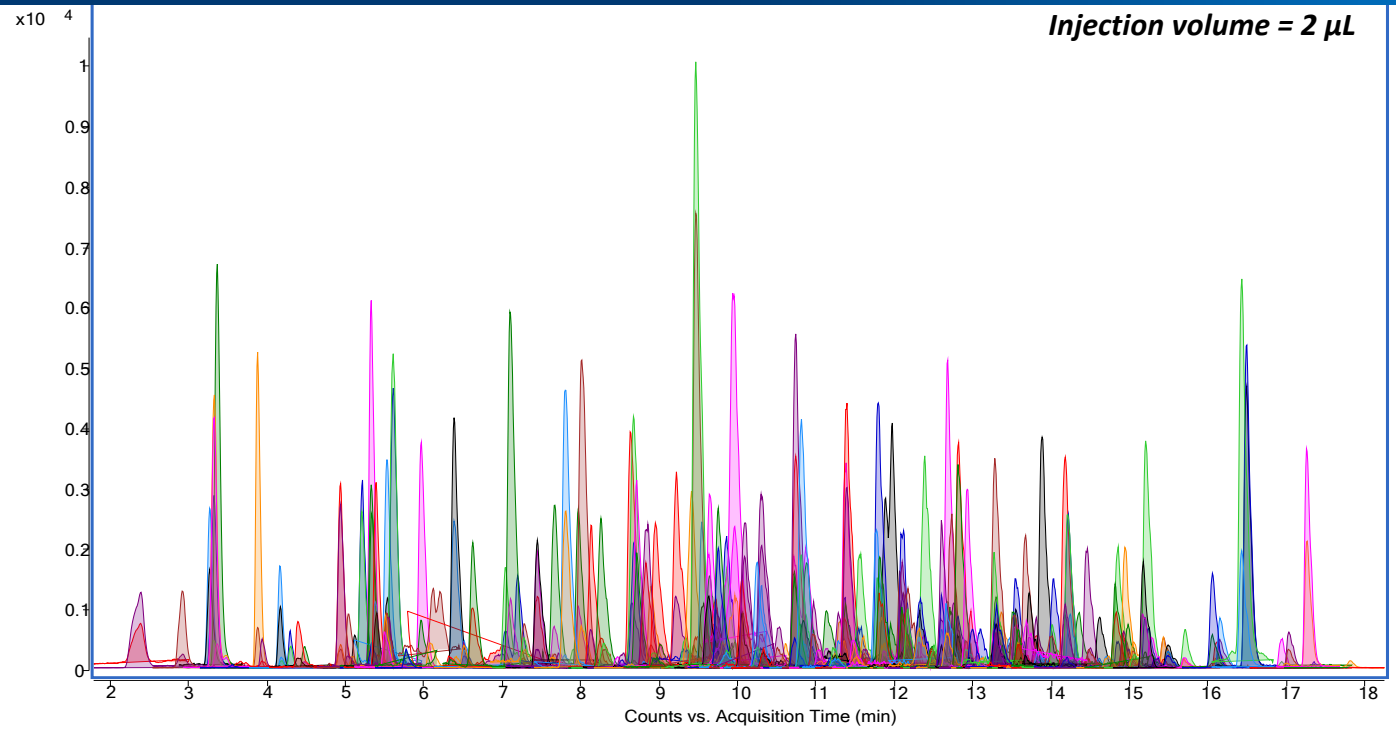


On



Multi-Residue Pesticide Analysis in Food

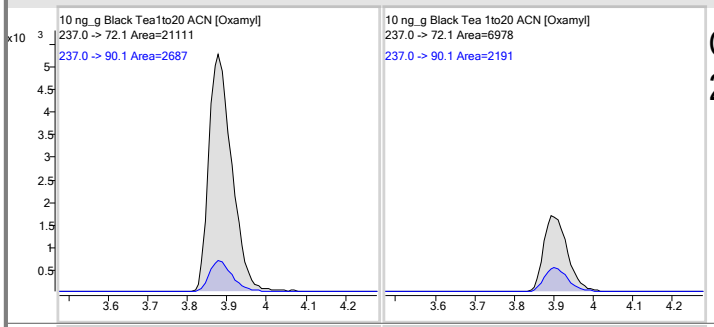
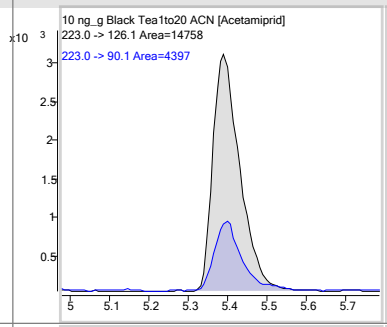
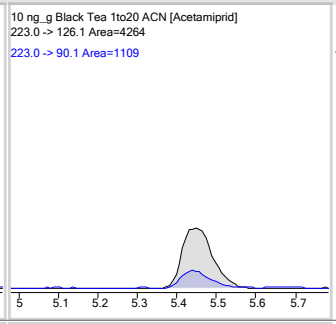
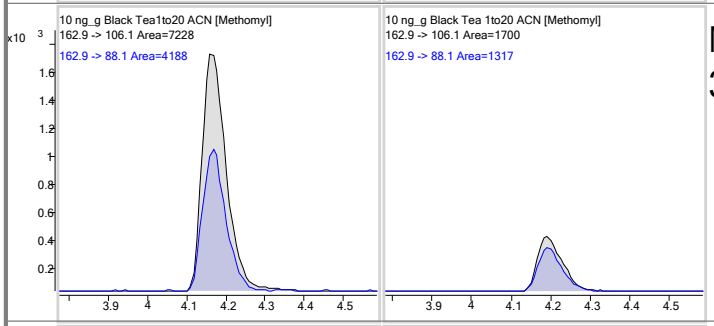
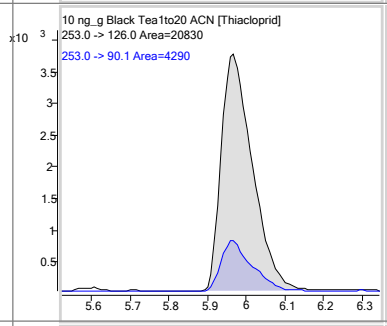
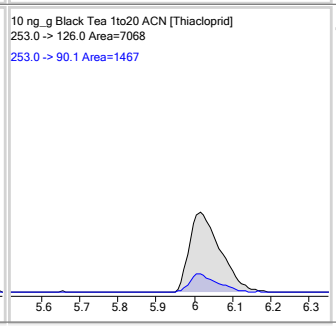
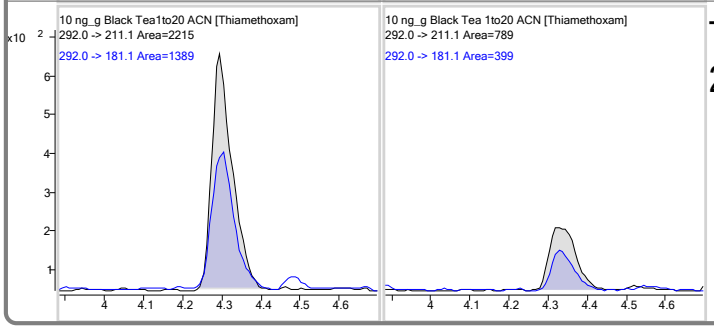
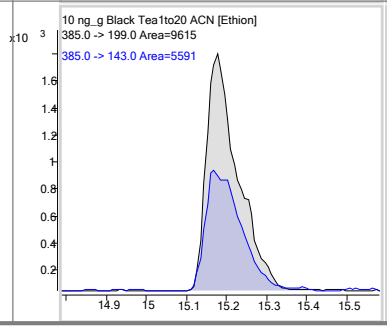
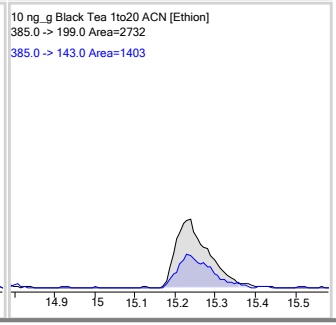
1:20 dilution of > 250 pesticides spiked into black tea at 10 ng/mL (ppb)



- Multi-residue pesticide analysis in food products – most demanding food safety applications
- Improved **sensitivity** and **precision** of the 6470 allows **accurate** quantitation of pesticides <Maximum Residue Limits (MRLs) imposed by EU, with higher degrees of sample dilution
- Sample dilution reduces matrix effects, improves method **robustness,** allows more efficient ionization and enables the use of solvent calibration with better **accuracy**

Sensitivity Improvements for High Relevance Pesticides

1:20 Dilution of 10 ng/mL of Pesticides in Black Tea, Peak Area 6470 vs. 6460

6470	6460	Area Gain	6470	6460	Area Gain
		Oxamyl 2.8x			Acetamiprid 3.4x
		Methomyl 3.9x			Thiacloprid 2.7x
		Thiamethoxam 2.5x			Ethion 3.2x

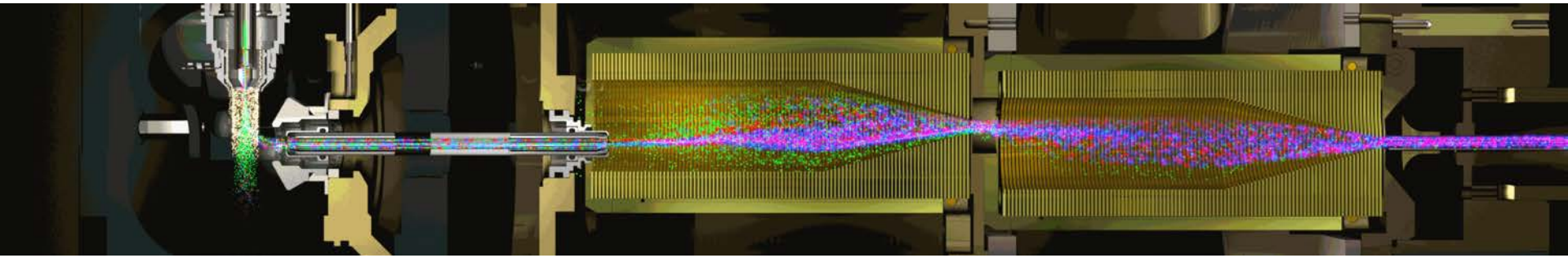
- Improved **sensitivity** (average peak area gain of **3.0x**) are observed on the new **6470** vs. **6460**

Recoveries in Black Tea with Different Dilutions

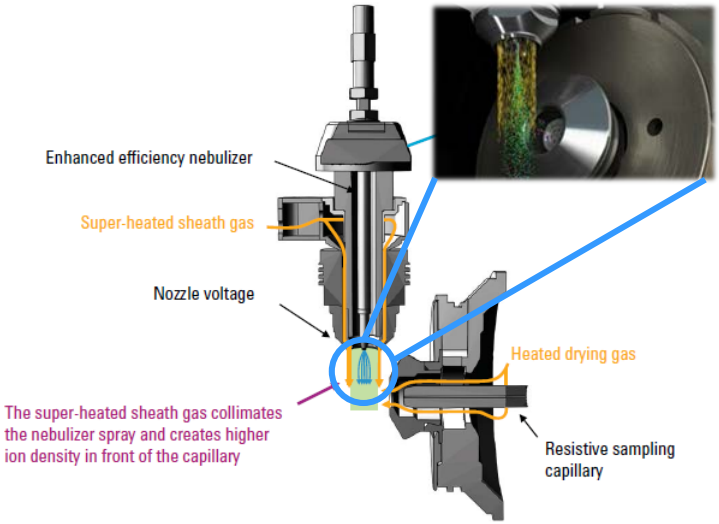
Pesticides	No Dilution	Dilution 1:2	Dilution 1:5	Dilution 1:10	Dilution 1:20
Acephate	59.5 +/- 0.7	70.5 +/- 1.2	77 +/- 1.3	74.8 +/- 0.8	83.5 +/- 2.4
Aldicarb	26.3 +/- 1.3	42 +/- 1.9	64.1 +/- 2.7	71.3 +/- 6.1	83.9 +/- 6
Carbofuran	45 +/- 0.3	60.5 +/- 0.4	73.1 +/- 0.6	73.3 +/- 0.7	82.9 +/- 0.7
Diethofencarb	86.2 +/- 1.4	92.8 +/- 1	89.1 +/- 1.1	80.4 +/- 1.7	86.7 +/- 2.1
Dimethoate	27.2 +/- 0.4	40.9 +/- 0.5	57.2 +/- 0.9	63.3 +/- 1.2	75.4 +/- 1.9
Epoxyconazol	65.2 +/- 1	71.9 +/- 1.6	77 +/- 2.3	75.3 +/- 3.1	84.6 +/- 7.3
Ethion	46.7 +/- 0.8	66.7 +/- 0.9	82.7 +/- 1.8	78 +/- 1.4	85.1 +/- 5.3
Flufenoxuron	92.3 +/- 1	93.1 +/- 2.7	89.6 +/- 3	80.2 +/- 5.4	87.1 +/- 10.6
Methamidophos	44.9 +/- 0.8	56.5 +/- 0.2	66 +/- 0.4	68.4 +/- 0.4	78 +/- 1.1
Methidathion	66.2 +/- 1.8	76.7 +/- 0.9	82 +/- 3.3	83.9 +/- 2.2	82.7 +/- 10.9
Methomyl	11.7 +/- 0.2	25 +/- 0.3	45.4 +/- 0.9	56.8 +/- 1.2	70.2 +/- 1
Oxamyl	15.7 +/- 0.2	27.8 +/- 0.3	47.6 +/- 0.3	58.5 +/- 0.6	71.8 +/- 0.6
Pirimicarb	50.2 +/- 0.3	62.6 +/- 0.4	72.6 +/- 0.3	71.5 +/- 0.8	80.3 +/- 0.6
Pyridaben	51.1 +/- 0.5	62.7 +/- 0.3	71.2 +/- 0.7	70.5 +/- 0.8	79.1 +/- 0.8
Thiacloprid	22.6 +/- 0.1	35.2 +/- 0.5	52.1 +/- 0.4	58.7 +/- 0.8	71 +/- 1.2

- Green cells show **recoveries** of 70 – 120%, which are in full compliance with SANCO requirements
- Pesticides achieved acceptable recoveries (70-120%) and less signal suppression with 1:20 dilution

For more sensitivity – iFunnel based 6495B

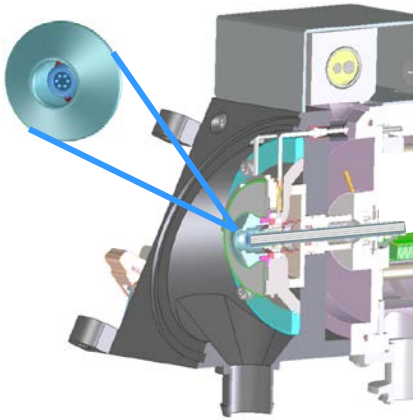


Agilent Jet Stream



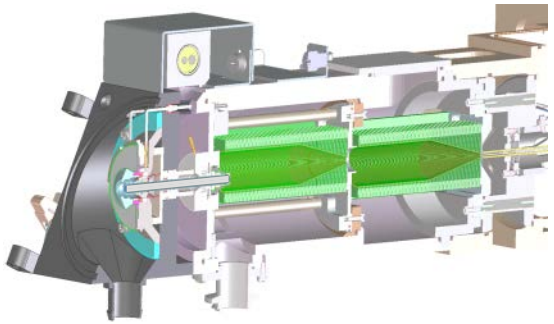
- Thermal gradient focusing
- Efficient desolvation
- Creates an ion rich zone

Hexabore Capillary



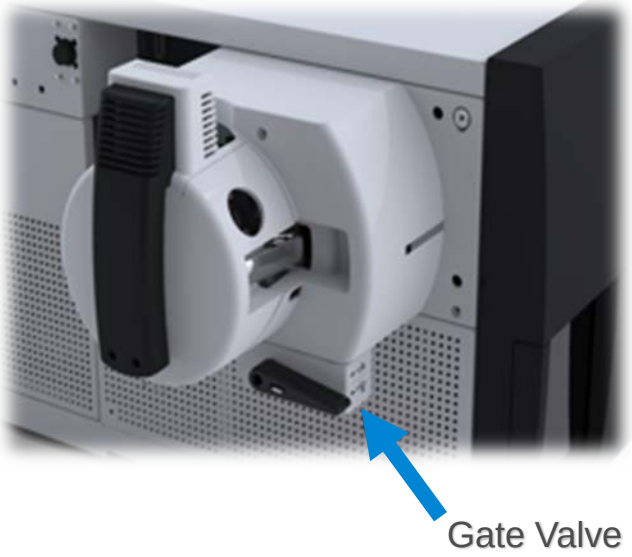
- Six capillary inlets
- Samples x10 times more ion rich gas

Dual Ion Funnel

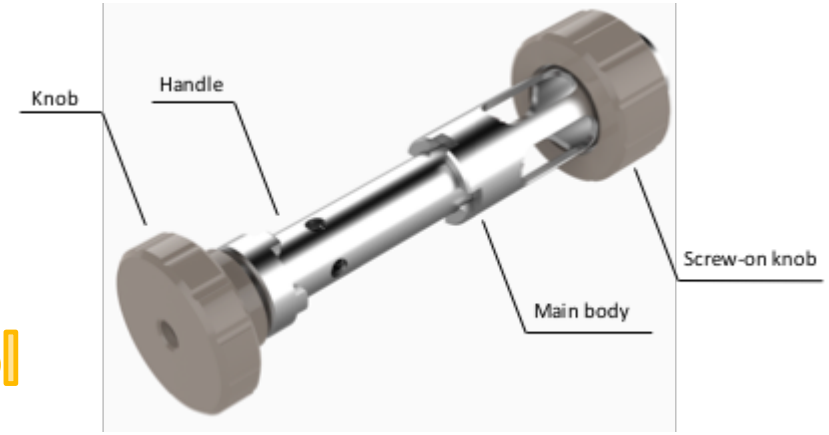


- Removes the gas but captures the ions
- Removes neutral noise

For more sensitivity – iFunnel based 6495B Gate-Valve Assembly added for ease of use



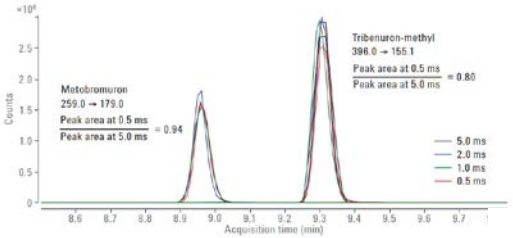
Gate Valve Assembly for the 6495B

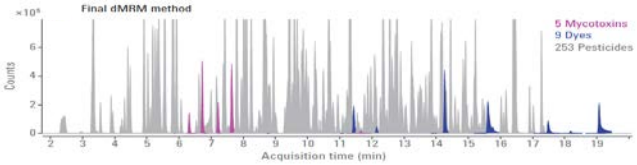


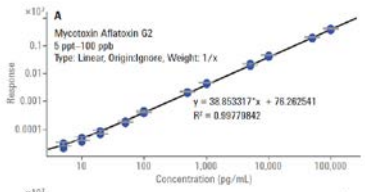
Capillary Puller Tool

Applications at Launch: Food

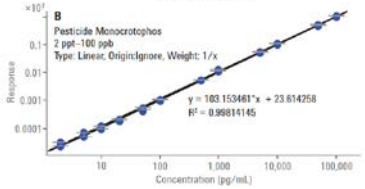
Featuring Triggered MRM, Fast Polarity Switching, and 0.5 ms dwell times



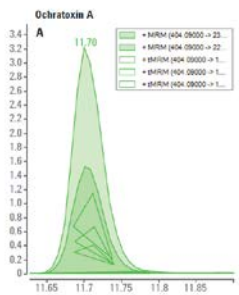


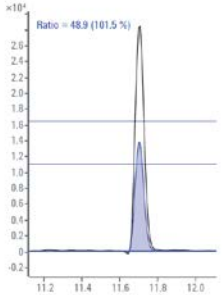


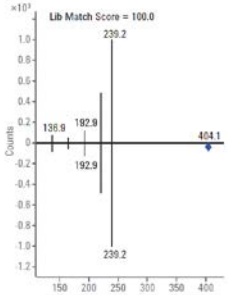
Mycotoxin	LLOQ (ppt)	IDL (ppt)
Aflatoxin B1	10	3.7
Aflatoxin B2	20	2.2
Aflatoxin G1	5	2.3
Aflatoxin G2	5	2.6
Ochratoxin A	500	274.5

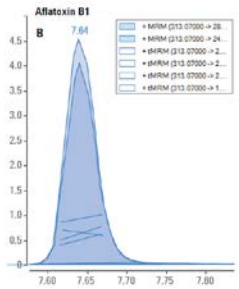


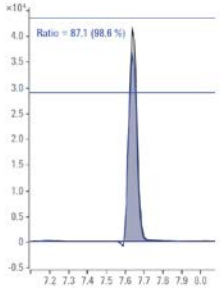
Dye	LLOQ (ppt)	IDL (ppt)
Dimethyl yellow	50	7.9
Orange II	1,000	733.5
Rhodamine B	500	294.1
Sudan I	50	24.0
Sudan II	50	8.8
Sudan III	500	158.8
Sudan orange G	500	190.5
Sudan red 7B	50	11.6
Sudan red G	100	24.1

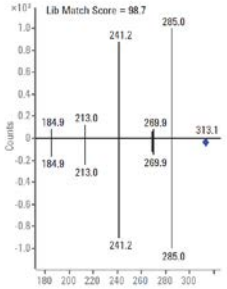












Know your LC System:

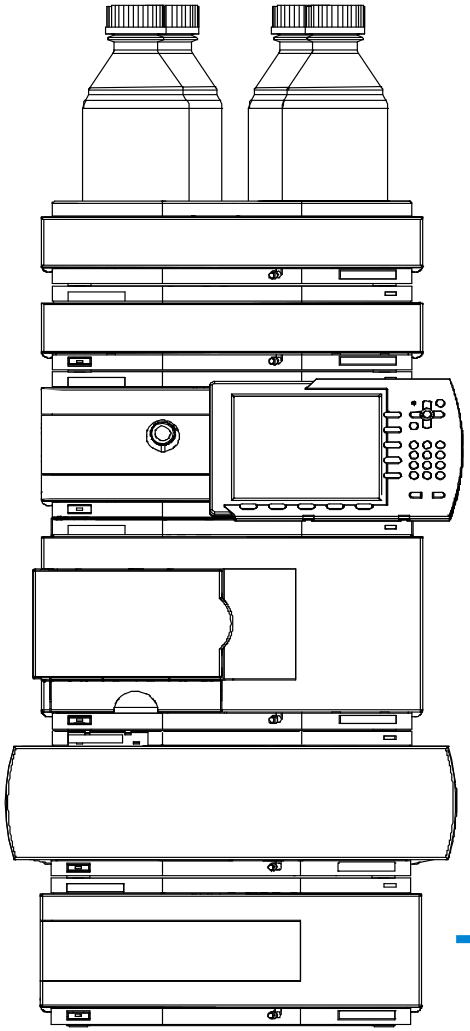
Vacuum Degasser

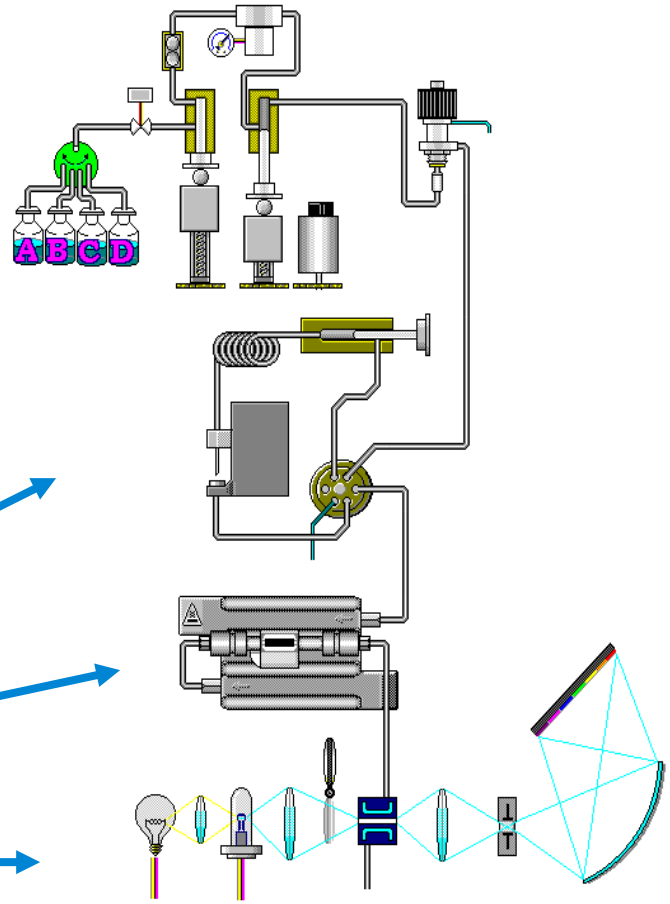
Pump

Autosampler

Column Comp.

Detector

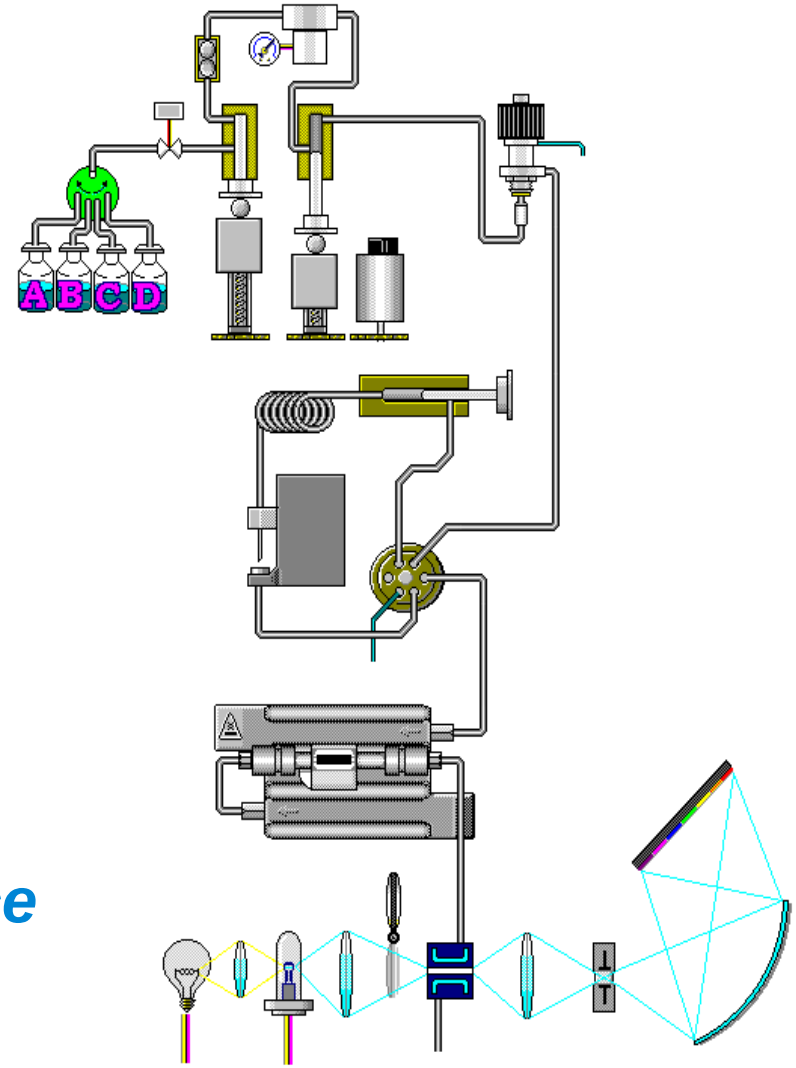




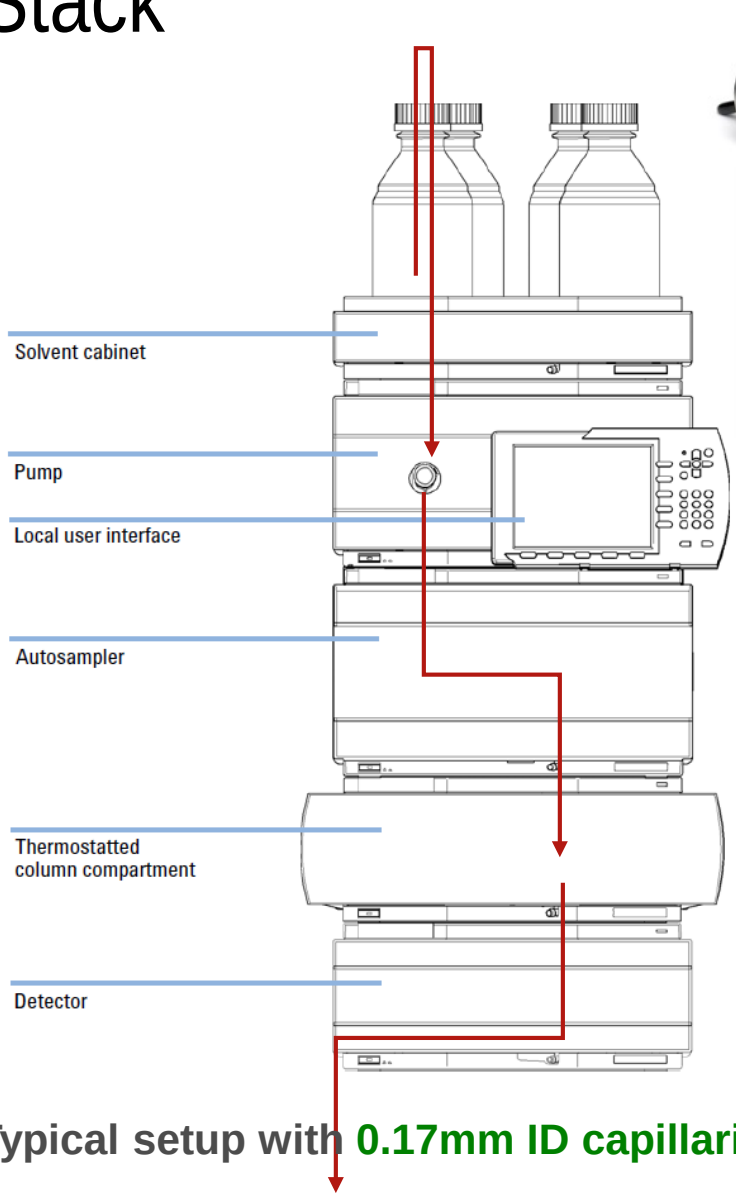
Know Your HPLC Flow Path:

- ✓Where are the moving parts?
- ✓Where can blockages to flow occur?
- ✓Where are the consumables that need to be replaced on a regular basis (PM)?
- ✓Where can leaks occur ?

What can I do to eliminate, reduce or anticipate potential problems with the LC ?



The HPLC Stack



Typical setup with 0.17mm ID capillaries

Best Practices start with Maintenance

Daily tasks

- Replace mobile phase based on water/buffer.
- Replace organic mobile phase every other day.
- Check seal wash solvent.
- Run conditioning with composition of your application.

Weekly tasks

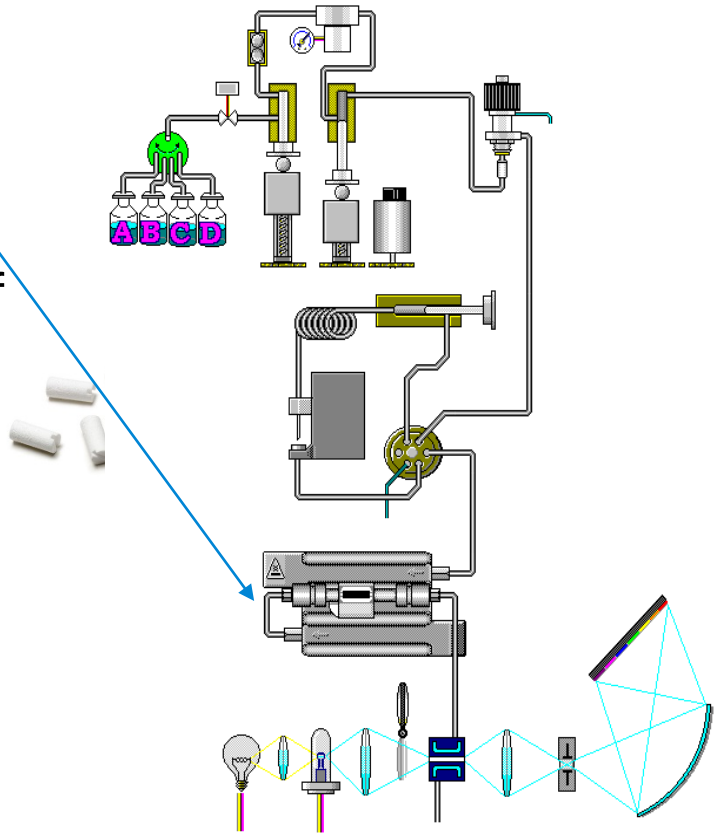
- Change seal wash solvent (10:90 isopropanol:water) and bottle.
- Flush all channels with water to remove salt deposits.
- Visually inspect solvent filters. Clean or exchange if necessary.

Best Practices start with Maintenance

- Use clean bottles only.
- Select solvent volume to be used up within 1 – 2 days.
- Use only HPLC- grade solvents and water filtered through 0.2 µm filters.
- Label bottles correctly with bottle content, and filling date / expiration date.
- Use solvent inlet filters.
- Reduce risk of algae growth: use brown bottles for aqueous solvents, avoid direct sunlight.

Filters and Bottle necks for blockages

- **Solvent inlet** filters in solvent bottles
  glass: 20um – replace if needed!
  SST: 12-14um – replace, opt sonicate
- **Heat exchanger** (bent? Connection?)
- **PTFE frit** in the purge valve at outlet of the *quaternary* pump head: replace
- *Binary* pump only: **inline filter** at outlet check valve
- **Troubleshoot:** Disconnect other modules behind pump



Examples: used / unused Filters

Glass filters: 3150 - 0944

Stainless Steel Filters: 01018 – 60025

(less volume, no Na+ ions)

When to use purge, prime, condition ?

Purge

Change solvents

When pump is refilled with new/different mobile phase the purge valves allows both pump heads (binary pump) to be connected to waste at the same time

Prime

When the pump is dry

When Purge and Condition still show pressure ripple

Condition

When first starting up for the day or after changing solvents

When pump pressure ripple or composition ripple is too high (mixing noise) air bubble is hidden in pump head (listen)

best once a day to condition for smooth operation

How to prime when changing solvents

When changing solvents that are not miscible or incompatible (prevent precipitation of buffers etc)

1. Replace the column with a ZDV fitting.
2. If the channel is not filled with buffer, proceed to step 5.
3. Place solvent frit into water.
4. Flush the channel at appropriate flow for tubing (3-5 ml/min) for approximately 10 min or *~40ml*.
5. Modify the flow path if necessary for the new application.

→ *skip steps 6-8 for channels run with aq buffer*

6. Replace solvent bottle with IPA (good intermediate solvent).
7. Flush the channel with the same flow rate for ~ 20 ml.
8. Swap the IPA with your new solvent.
9. Repeat steps 1-8 for the other channels of the pump.
10. Install the column and equilibrate with your starting method conditions.

Priming Solvents

Table 13 Choice of Priming Solvents for Different Purposes

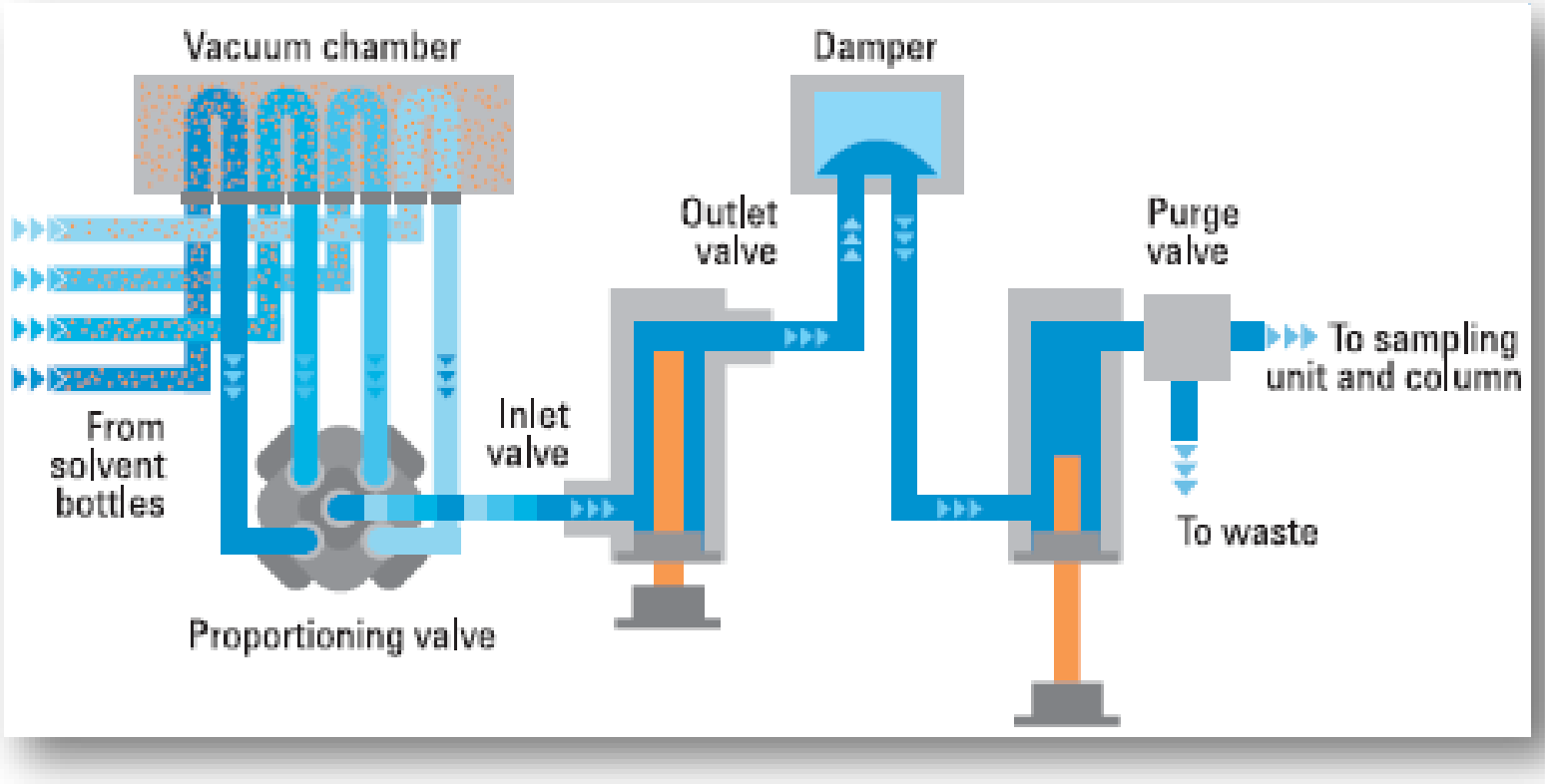
Activity	Solvent	Comments
After an installation When switching between reverse phase and normal phase (both times)	Isopropanol Isopropanol	Best solvent to flush air out of the system Miscible with almost all solvents
After an installation	Ethanol or methanol	Alternative to isopropanol (second choice) if no isopropanol is available
To clean the system when using buffers After changing aqueous solvents	HPLC grade water HPLC grade water	Best solvent to re-dissolve buffer crystals Best solvent to re-dissolve buffer crystals
After the installation of normal phase seals (P/N 0905-1420)	Hexane + 5% isopropanol	Good wetting properties

Performance Characteristics of the Pump

Important Characteristics	Influence on…
Common to isocratic and gradient pumps	
▪ Flow accuracy	• Retention time and peak area precision (system to system)
▪ Flow precision	• Retention time and peak area precision (within one system)
▪ Pressure pulsation	• Baseline noise
Common to gradient pumps only	
• Delay volume in low and high pressure mixing	• Gradient shape and precision
• Compositional accuracy	• Retention time and peak area precision (system to system)
• Composition precision	• Retention time and peak area precision (within one system)

Agilent 1260 Infinity Quaternary Pump

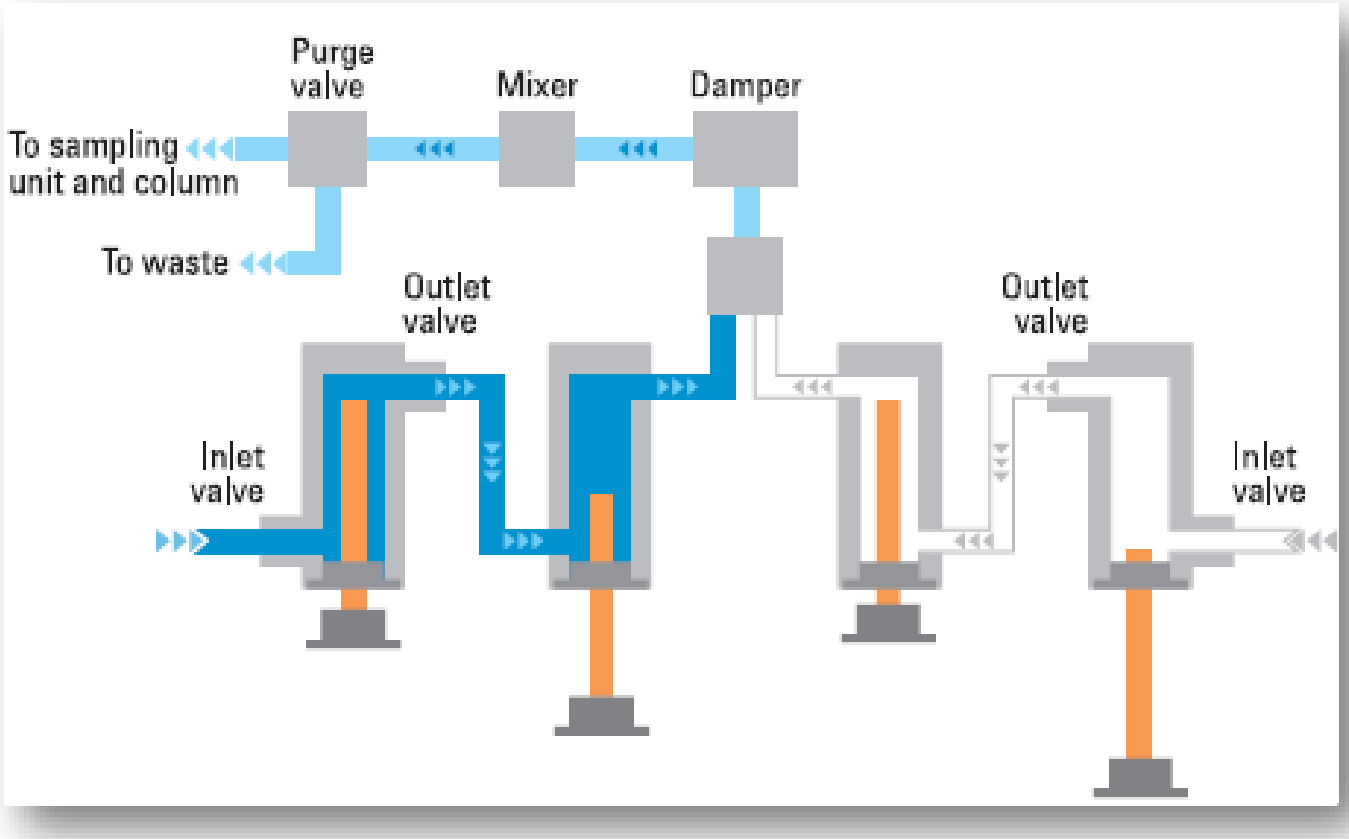
Low-pressure mixing (LPM) principle



Mixing by low-pressure proportioning valve before the pump head

Agilent 1260 Infinity Binary Pump

High-pressure mixing (HPM) principle



Combination and mixing of mobile phases <u>after</u> the pump head

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Option with Quaternary Pump Systems

Blending of buffer/modifier

Idea:

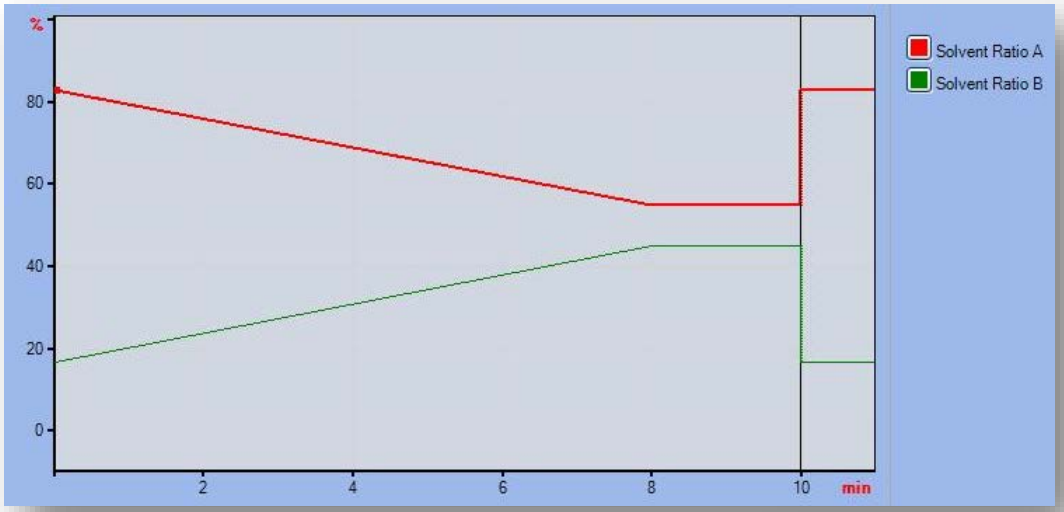
- One channel contains rather highly concentrated buffer/modifier
- This channel is blended into the gradient to generate the desired buffer/modifier concentration

Advantages:

- Only one, highly concentrated buffer/modifier solution has to be prepared, less errors
- Buffer/modifier concentration can be changed easily
- Buffer/modifier concentration becomes a method parameter

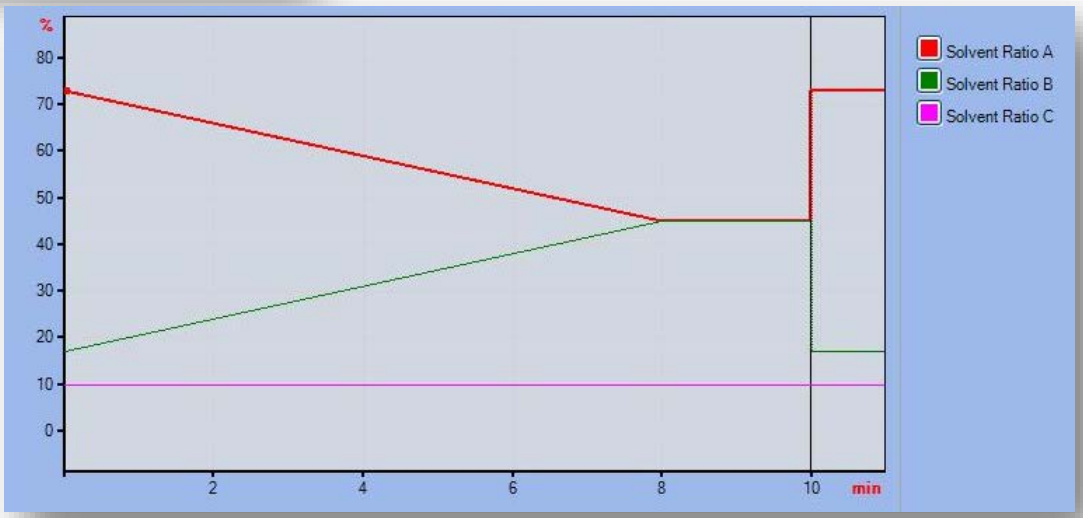
Agilent 1260 Infinity Quaternary Pump

Blending of modifier – Example: TFA



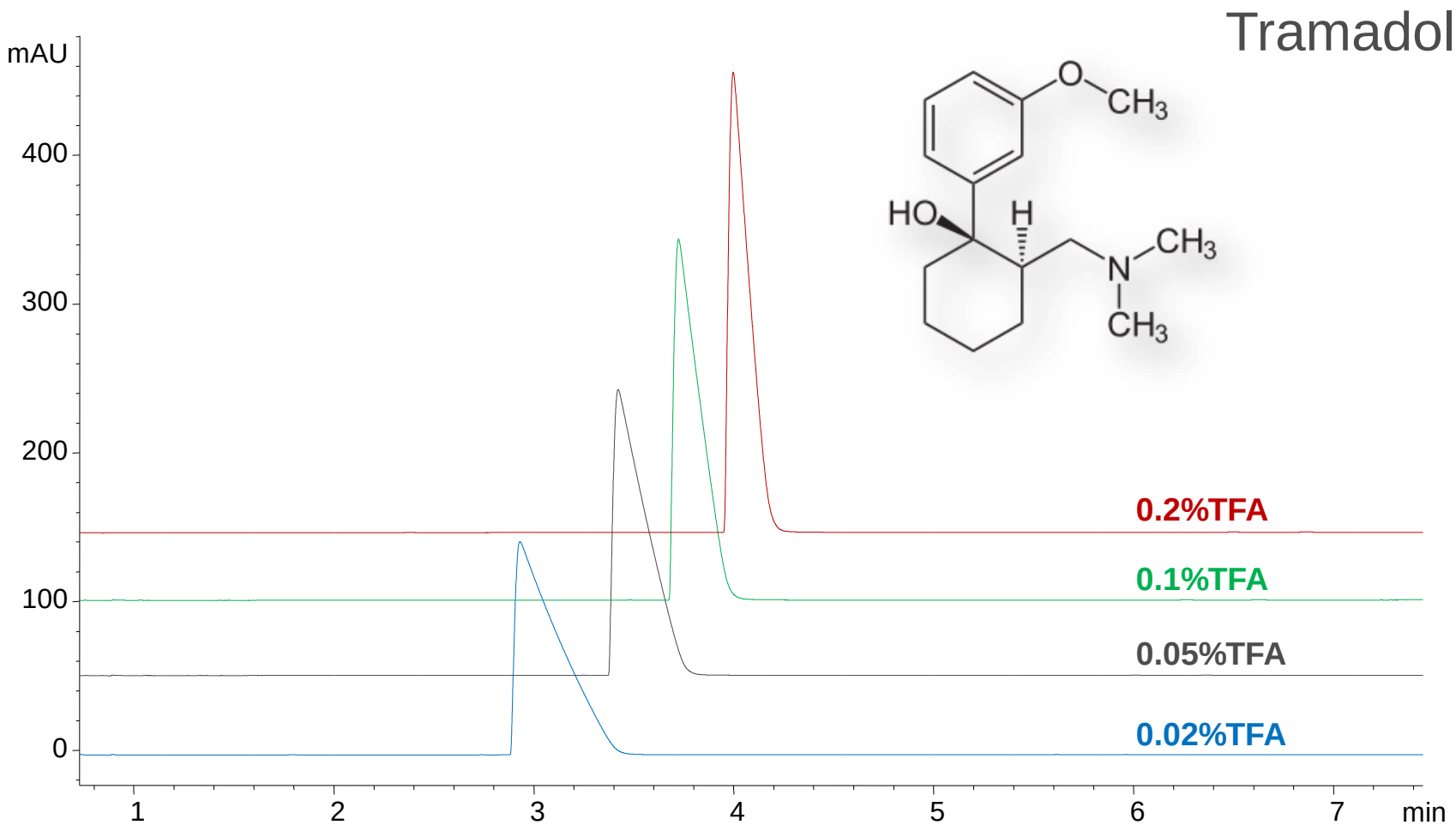
		A	B
At	0 min	83 %	17 %
	8 min	55 %	45 %

		A	B	C
At	0 min	**73** %	17 %	10 %
	8 min	**45** %	45 %	10 %



Agilent 1260 Infinity Quaternary Pump

Blending of modifier – Example: Tramadol and impurities



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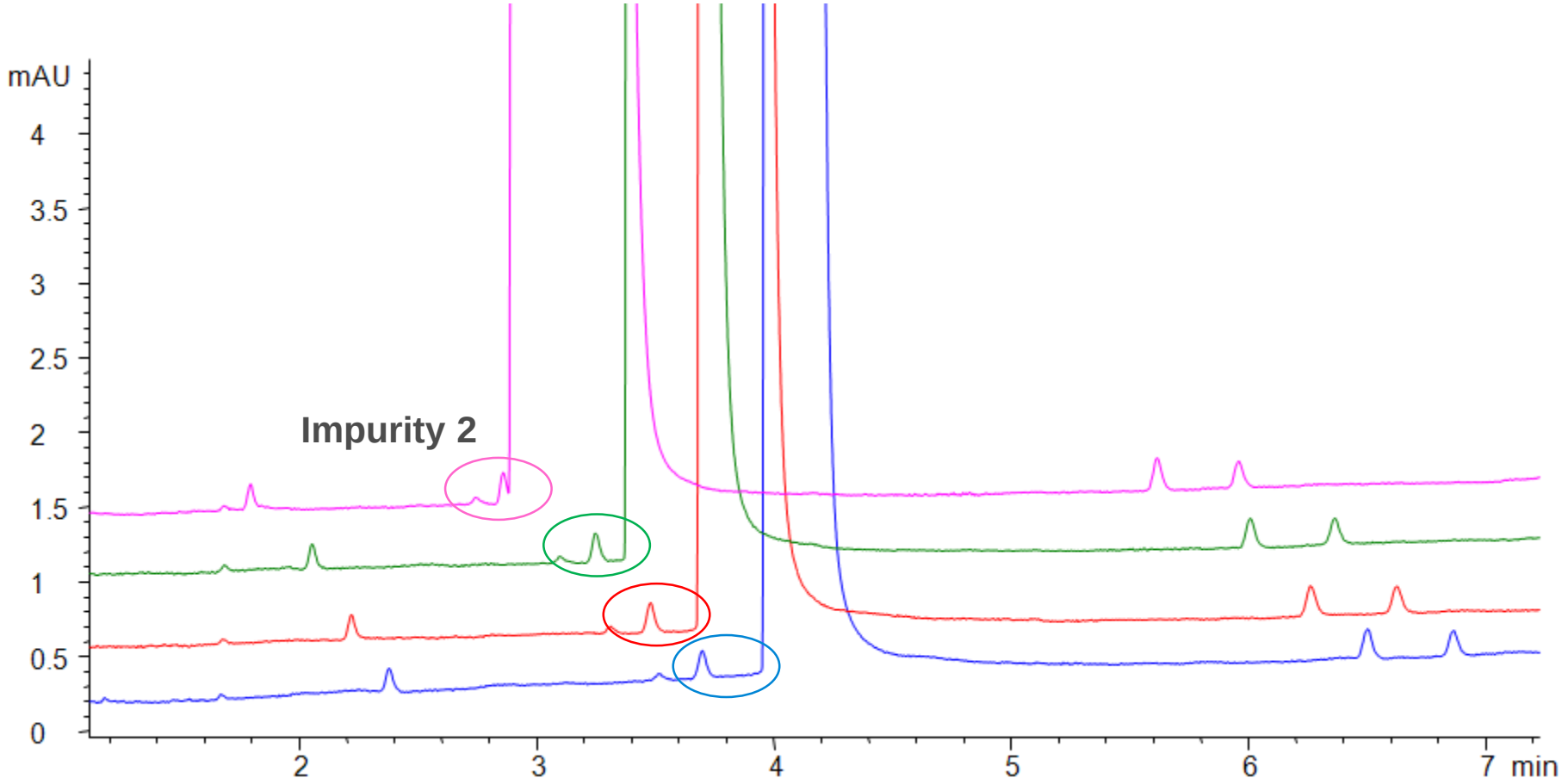
Agilent 1260 Infinity Quaternary Pump

Blending of modifier – Example: Tramadol and impurities



Agilent Technologies

Agilent 1260 Infinity Quaternary Pump

4 solvent channels, mixing of all 4 channels possible

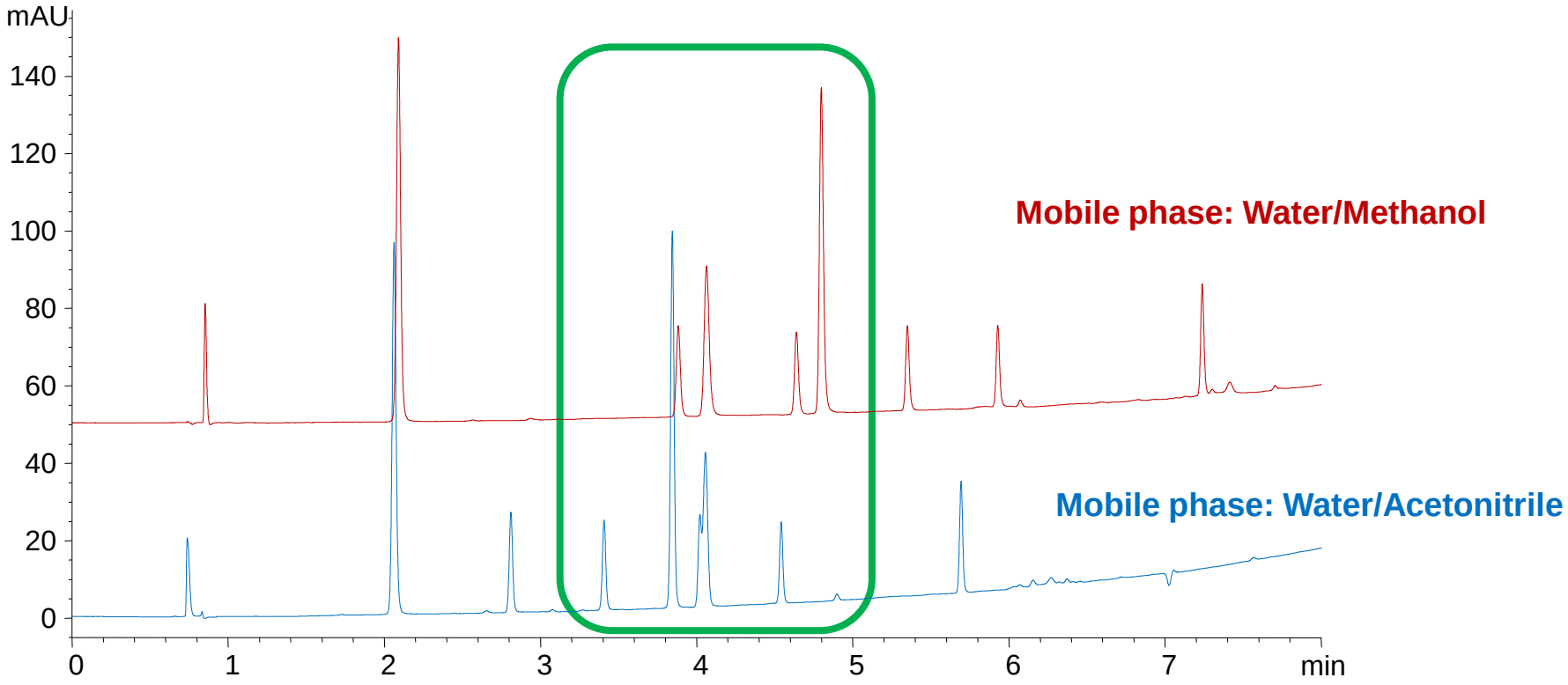
Applications:

- Solvent selection in Method Development
- Ternary or quaternary gradients
- Blending of buffers/modifiers

Agilent 1260 Infinity Quaternary Pump

Solvent selection in Method Development

Basic solvent selection, usually used on the organic side to select between acetonitrile and methanol



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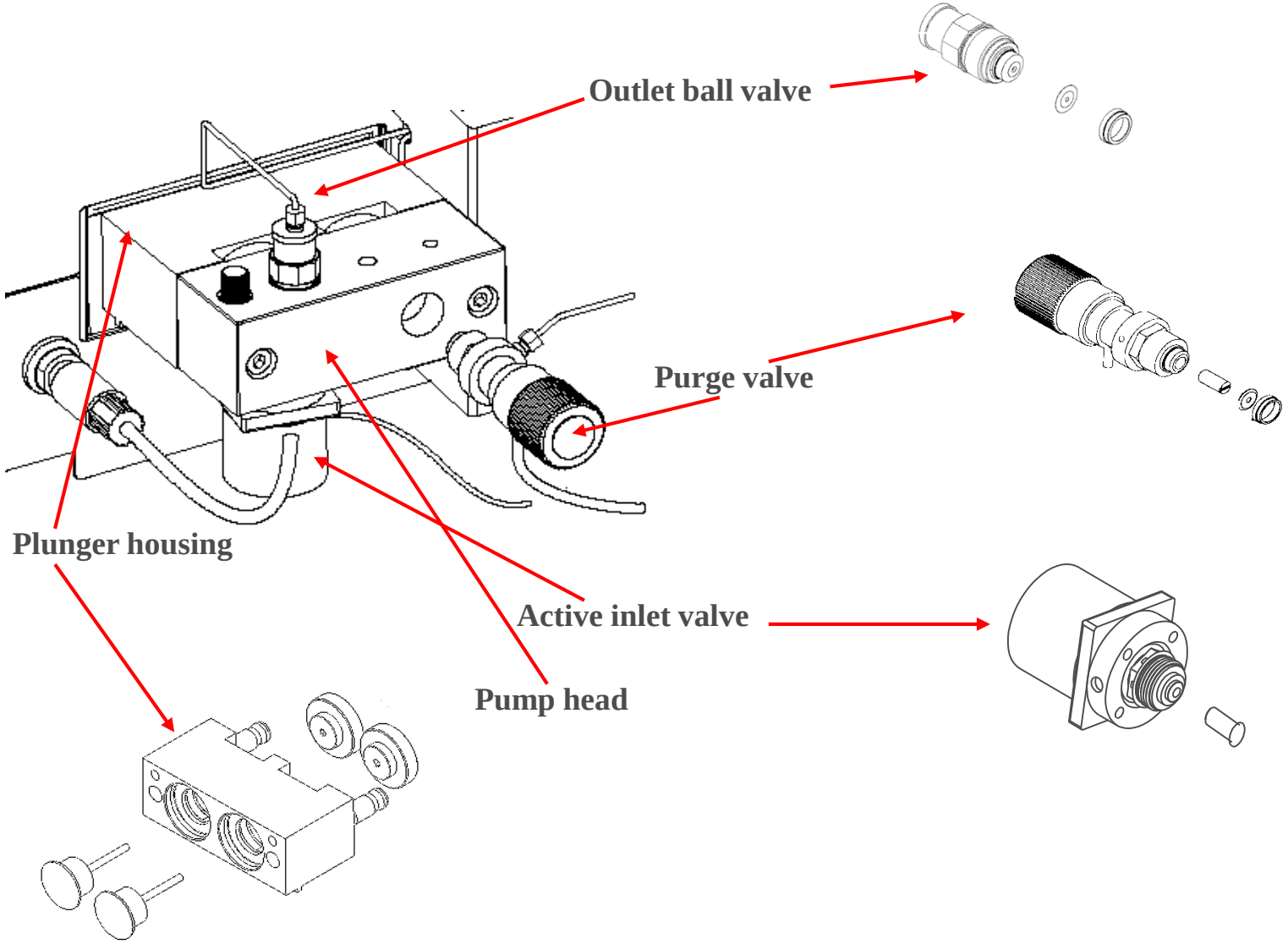
Pump and Degasser Maintenance

- Clean the degasser lines by flushing with isopropanol.
- When using buffers, flush with water, then with isopropanol.
- Check for air bubbles in outlet lines.
- Be aware of the possibility of microbial growth in aqueous phases
  - flush aqueous lines weekly with IPA
  - Don‘t top off water/solvents, use fresh bottles that have been cleaned with solvent and dried
- Check for solvent compatibility and flush with appropriate solvents
- Unused channels should be left in isopropanol.

Pump Head – Main Components



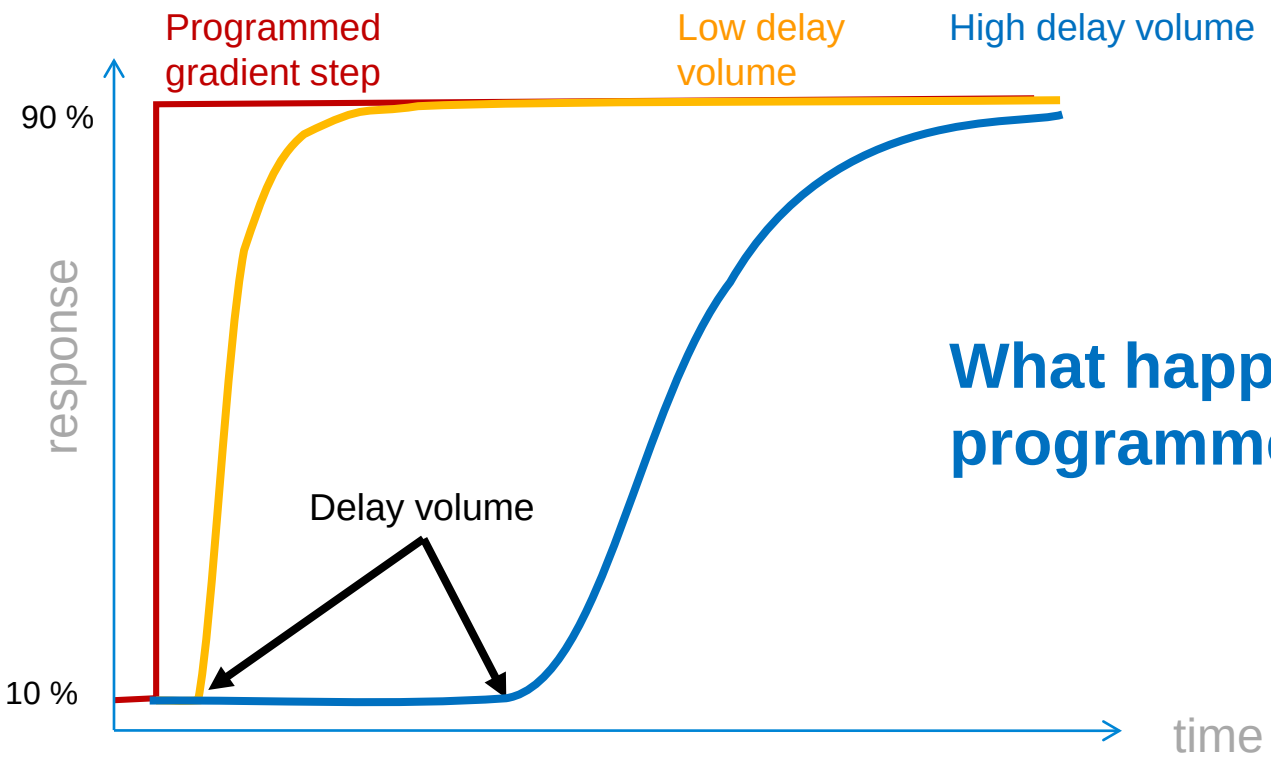
Seal Wash Option Operation

Maintenance & Routine

- Use 10% isopropanol in water
- No recycling of solvent
- Change solvent weekly (date on bottle)
- Position Wash bottle above pump
- Test peristaltic pump – use the prime and watch/feel for movement
- Material: PharmMed tubing
- Operate ***PERIODIC*** with 2min each 20min
- Setting in SW is **not a method parameter** – stays with pump (1290/InfII)

Delay volume

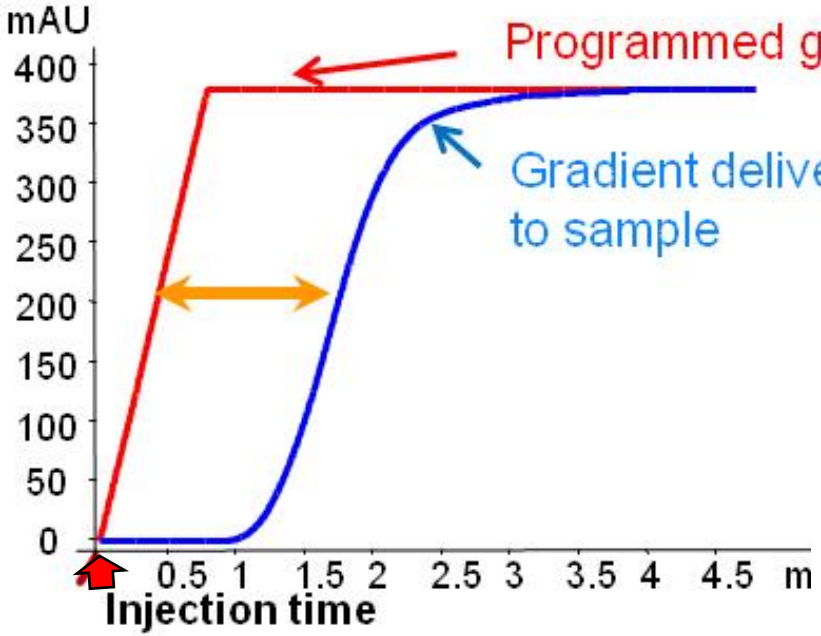
Impact of low delay volume



What happens with a programmed gradient?

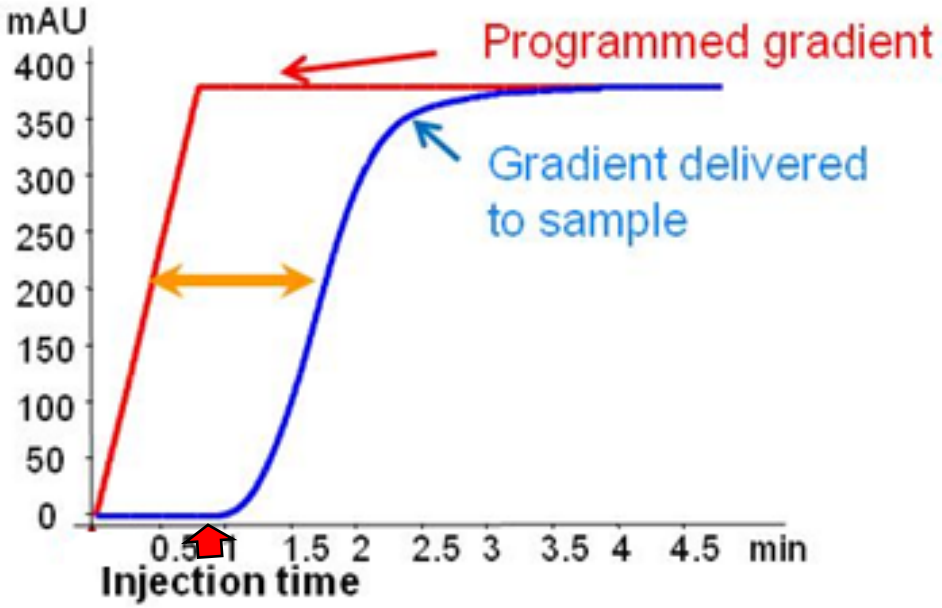
Total delay volume of the system (sum of capillaries, mixer, cells, valves..)

Effects of Delay Injection Program



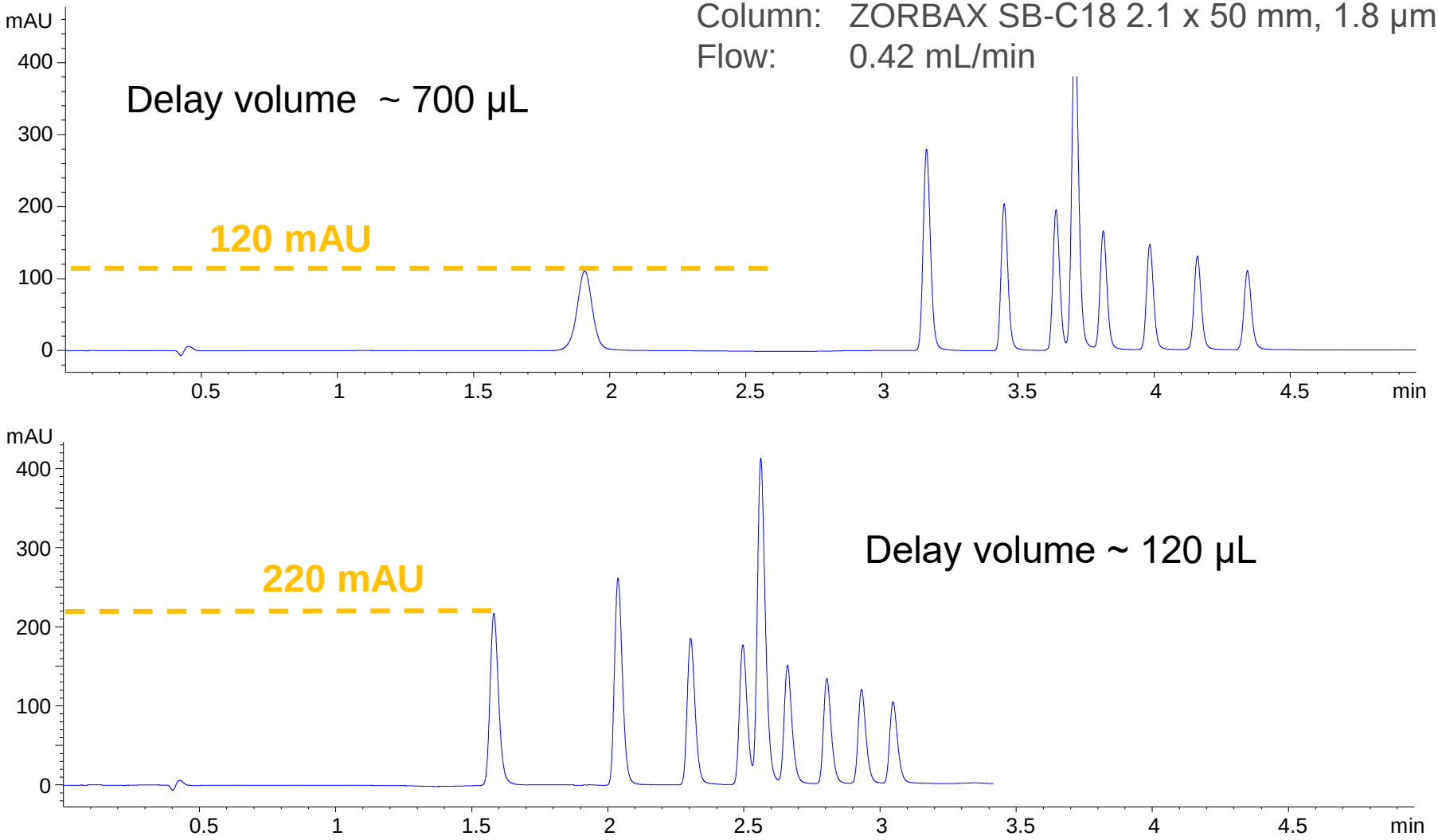
Standard Injection

Delayed Injection



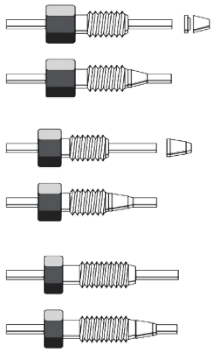
System – Signal height

System volumes – Delay volume

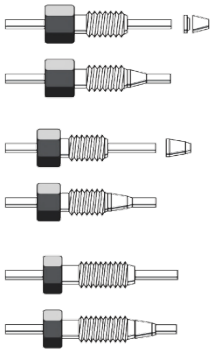


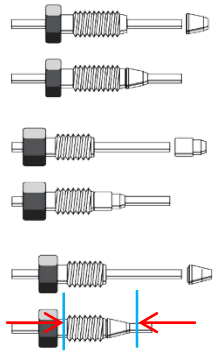
Don‘t mix your fittings!

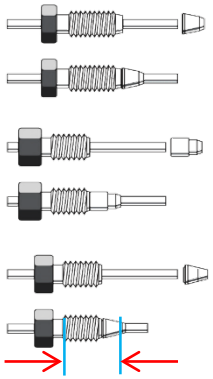
Swagelok



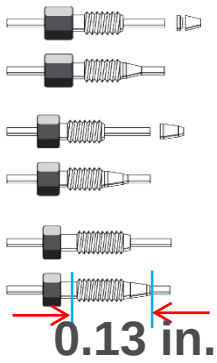
Parker



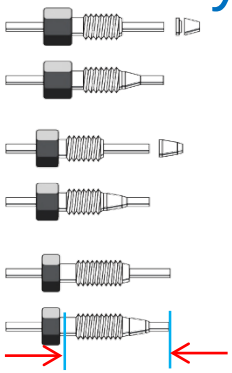


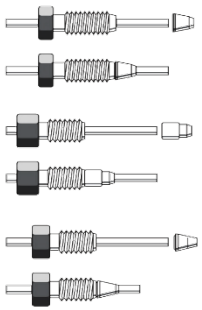


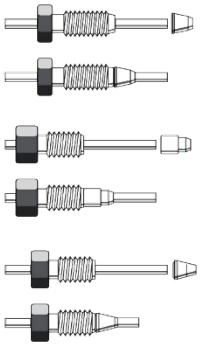
Waters



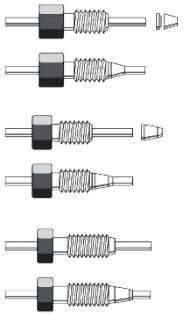
Rheodyne

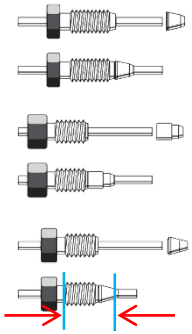




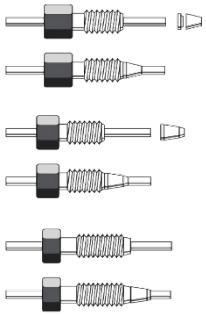


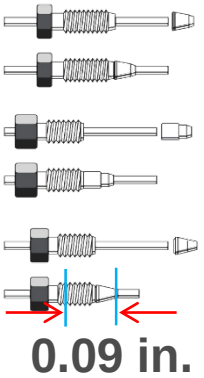
Valco





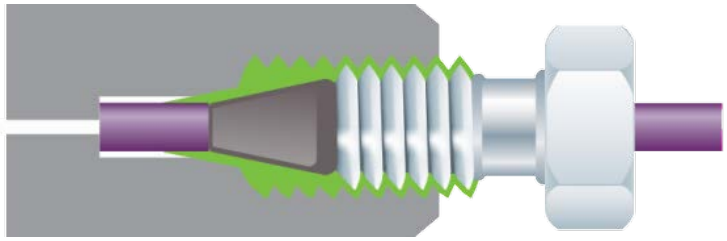
Upchurch



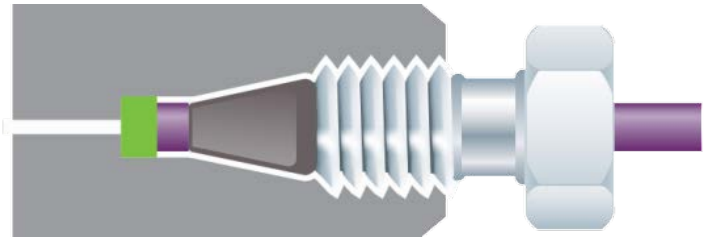


Comparison of correct and incorrect fitting connections

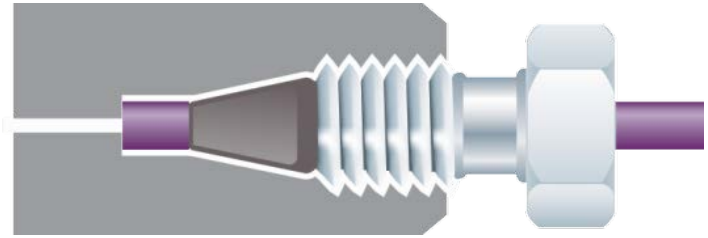
Stem length too long → leak

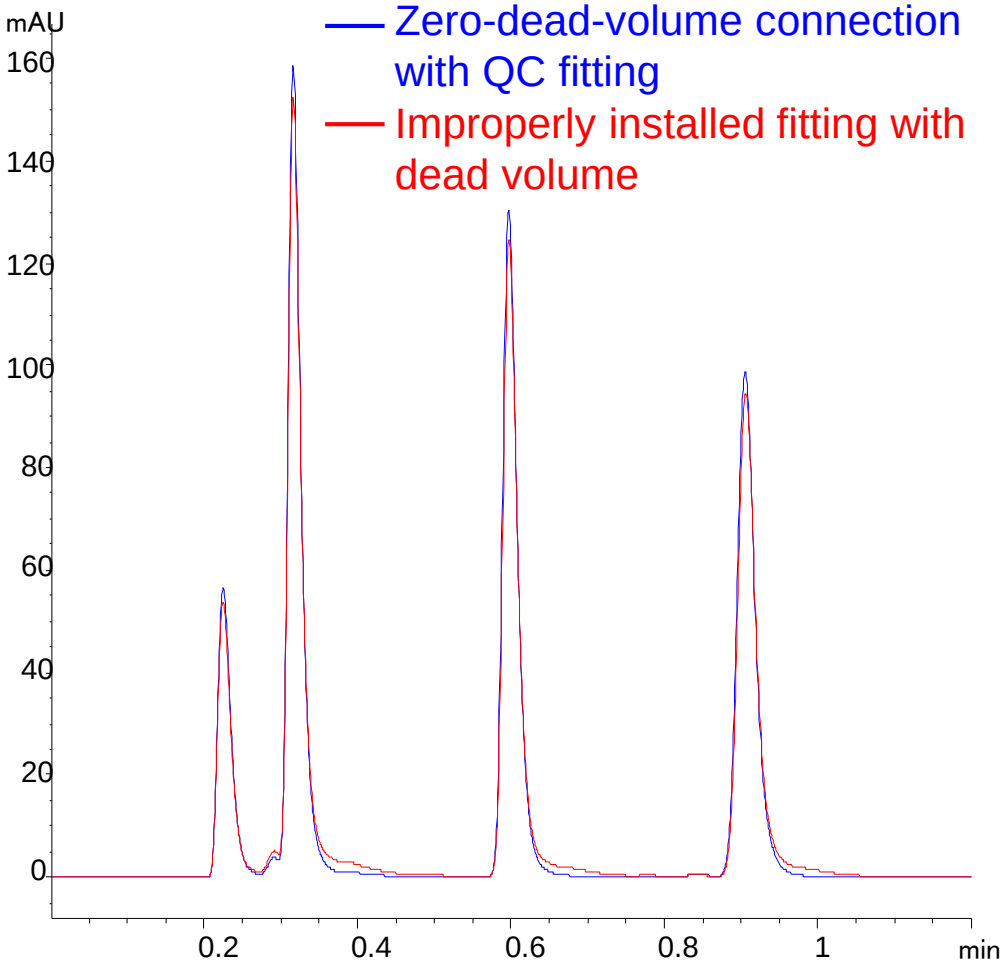


Stem length too short → dead volume

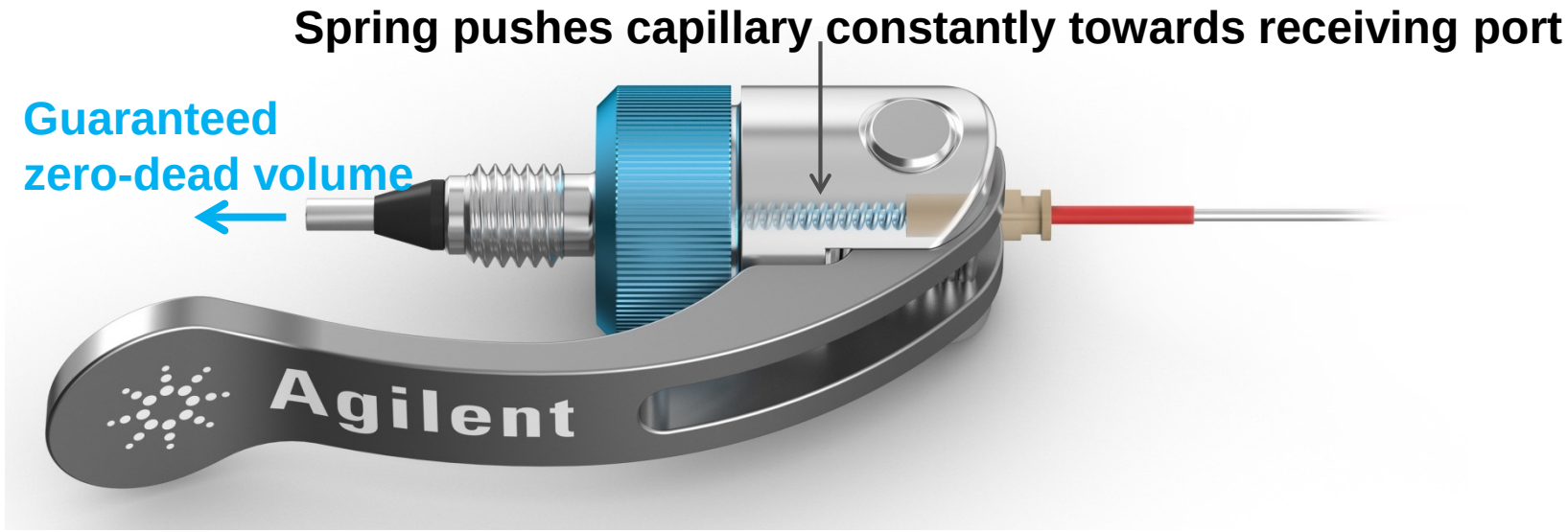


Properly fitted tubing, no dead volume



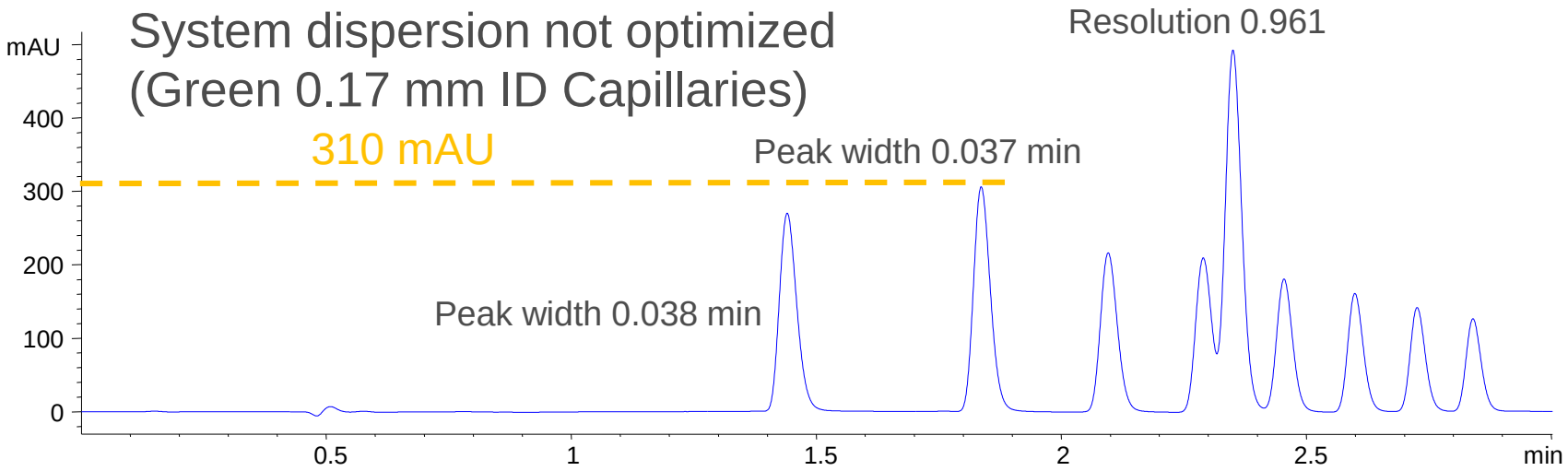


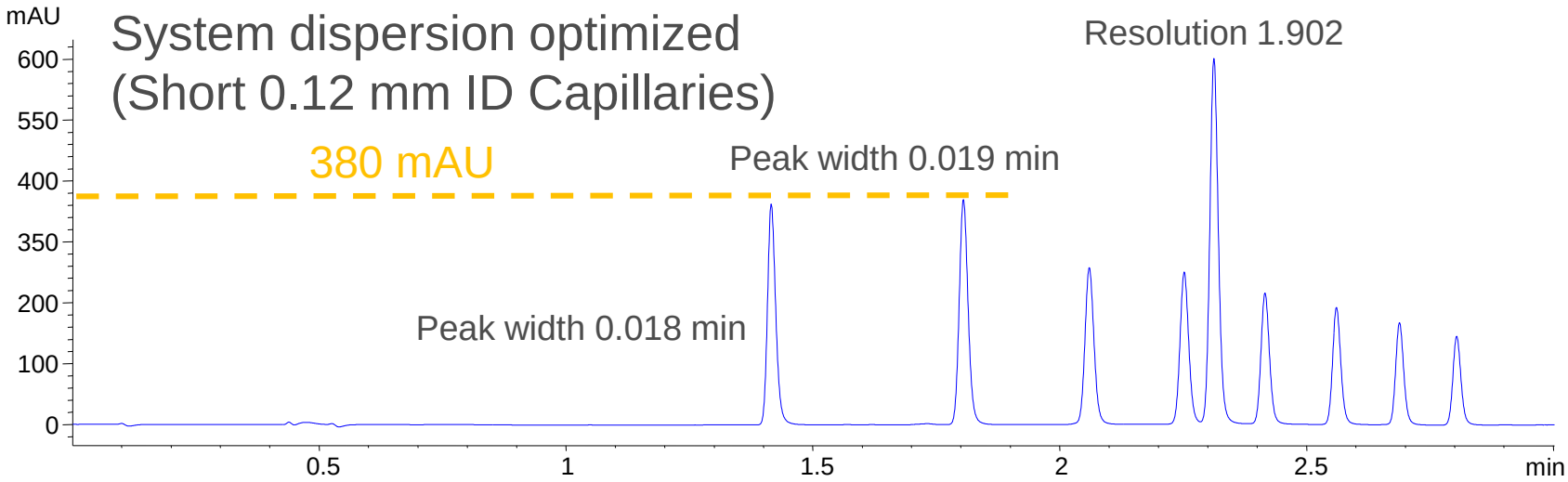
Designed-In Supplies for 1290 Infinity II
Agilent A-Line Quick Connect Fitting



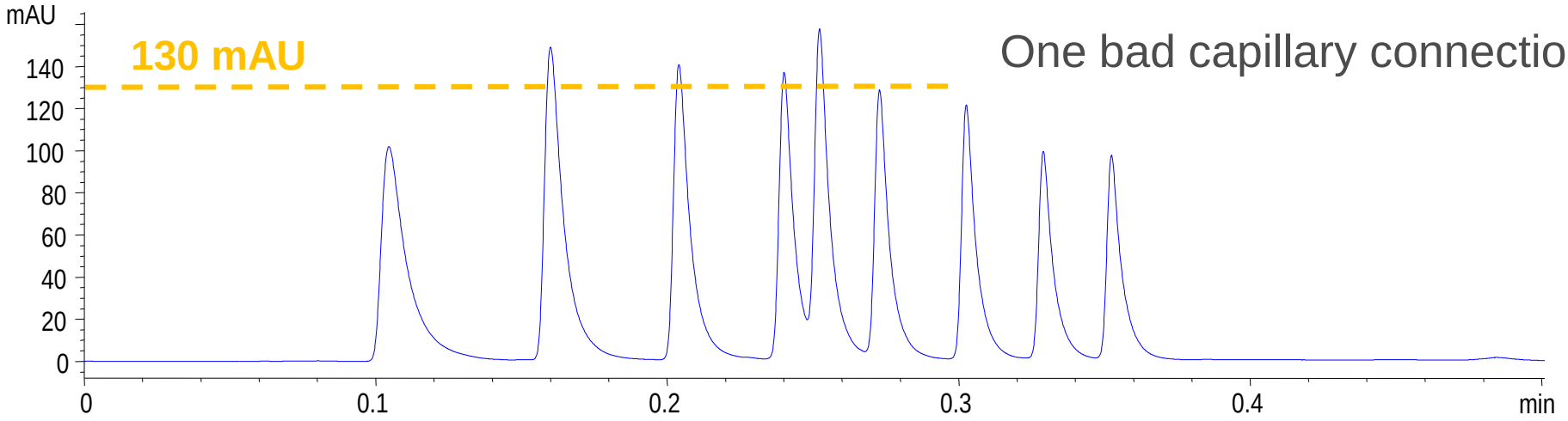
- Spring constantly pushes the tubing against the receiving port, delivering a reproducible connection with **no dead volume for consistent chromatographic performance**
- Spring assembly, including the lever, applies a constant force that presses the ferrule onto the tubing, so that **tubing slippage is avoided**
- **Compatible with all types of LC columns**
- **Little resistance** needed to tighten the fitting

System – Dispersion Optimization

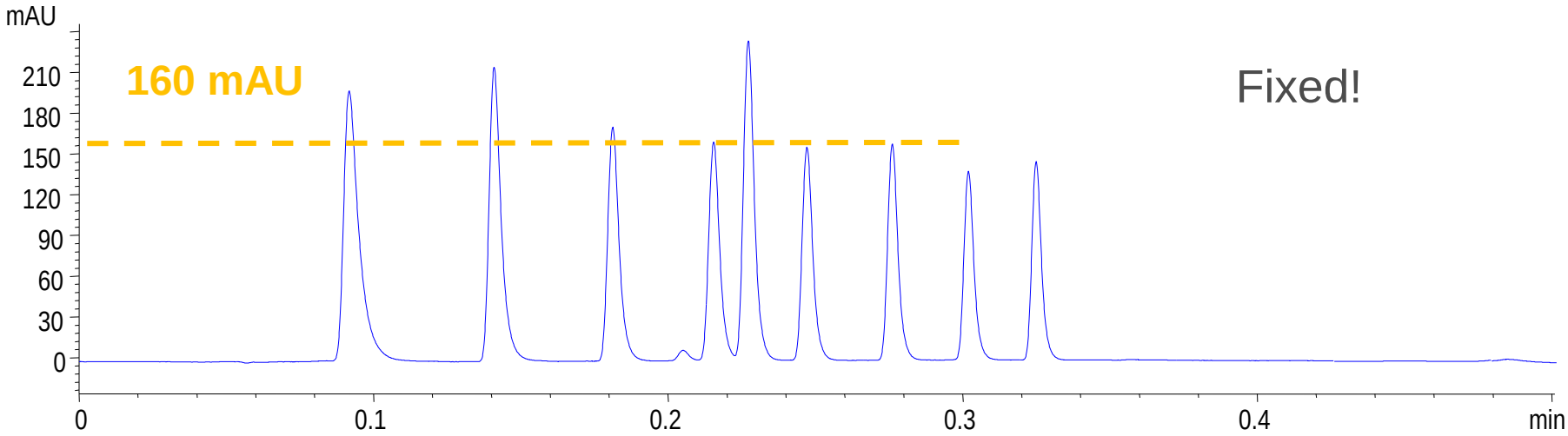




Influence post-column capillary connections



One bad capillary connection!

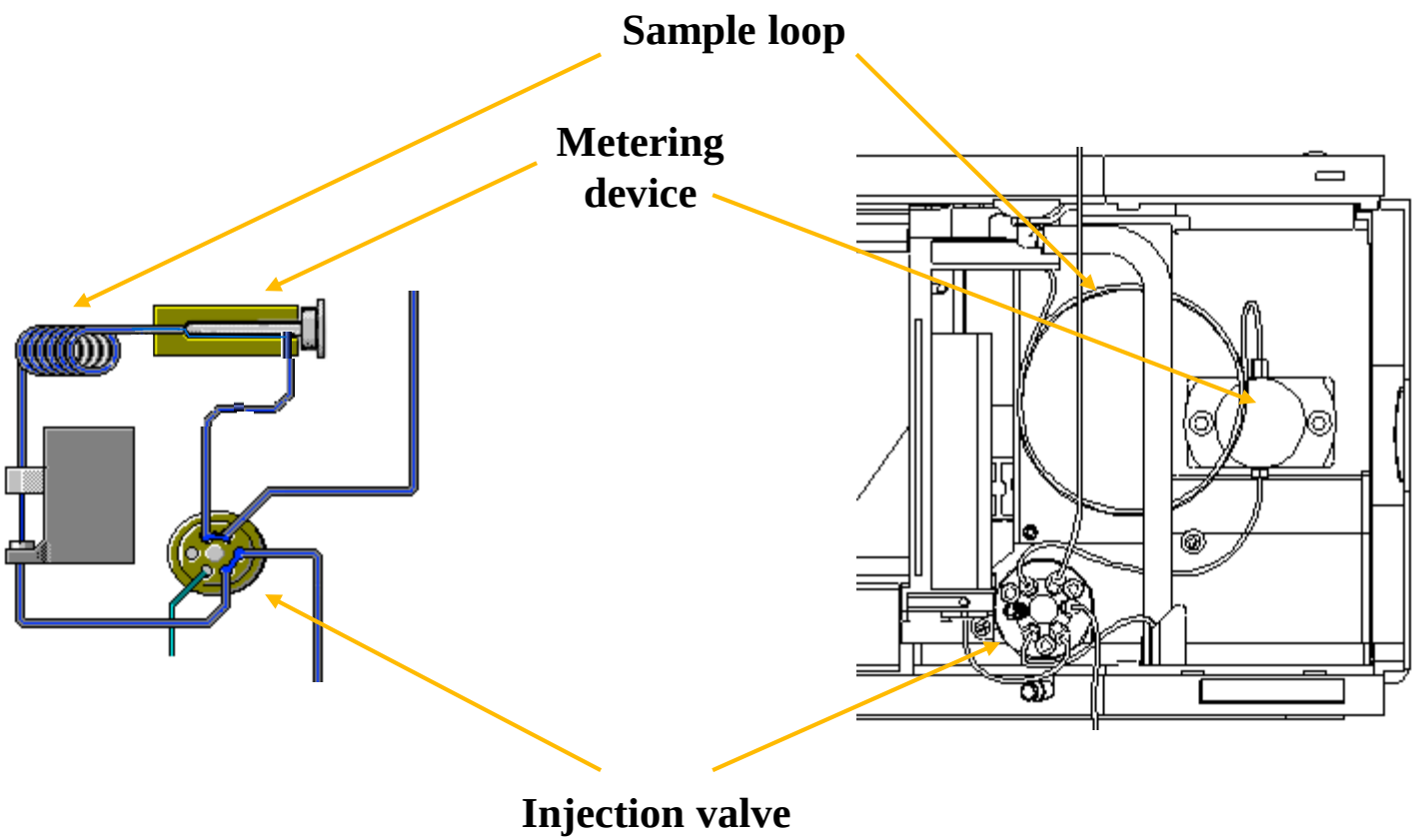


Fixed!

Performance characteristics of the Autosampler

Characteristic	Influences
Injection volume precision	Precision of peak area/height
Wide linearity	Accuracy of peak area/height (when using different injection volumes)
Minimum carry over	Precision of peak area/height
Wide dynamic injection volume	Versatility, application range

Schematic of Injection System



Autosampler procedure for reducing carry-over

General Recommendation to Lowest Carry-over

- For samples where needle outside cannot be cleaned sufficiently with water or alcohol use wash vials with an appropriate solvent. Using an injector program and several wash vials can be used for cleaning.

In case the needle seat has got contaminated and carry-over is significantly higher than expected, the following procedure can be used to clean the needle seat:

- Go to **MORE INJECTOR** and set needle to home position.
- Pipette an appropriate solvent on to the needle seat. The solvent should be able to dissolve the contamination. If this is not known use 2 or 3 solvents of different polarity. Use several milliliters to clean the seat.
- Clean the needle seat with a tissue and remove all liquid from it.
- **RESET** the injector.

Thermostatted Column Compartment

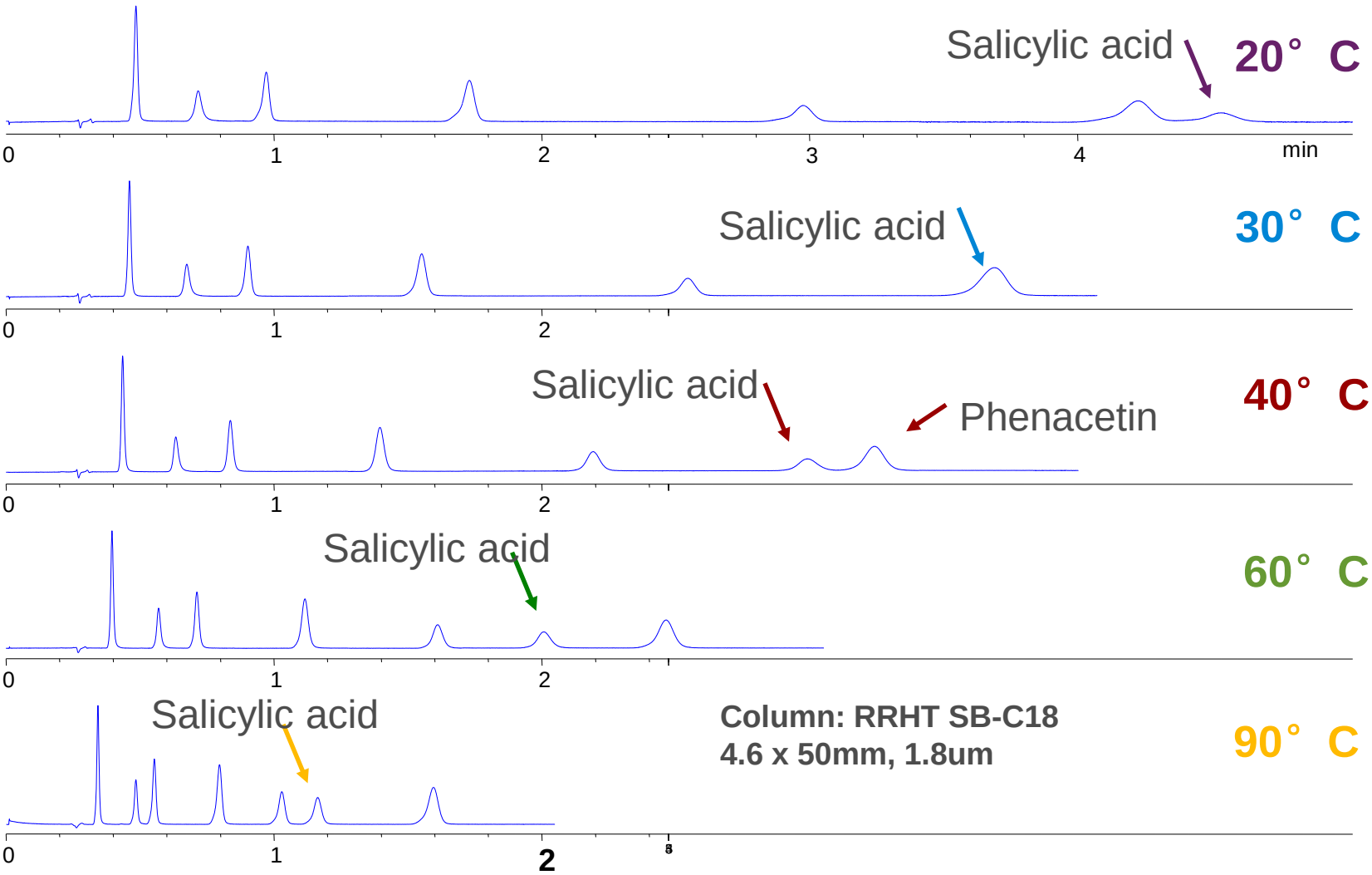
- **Important performance characteristics**

- **Excellent temperature accuracy** →
  - Elution order
  - Peak identification

- **Excellent temperature precision** →
  - Elution order
  - Retention time precision
  - Peak identification

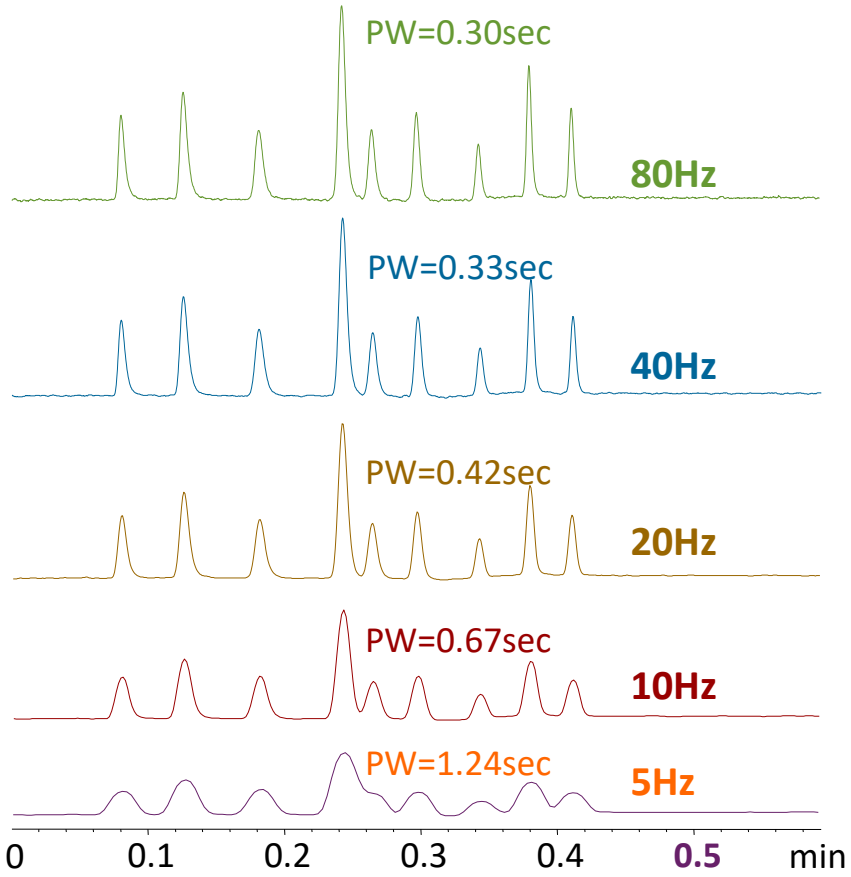
- **Influence on…**

Effect of Temperature on Separation



Conditions: A: 0.1% formic acid B: ACN w/ 0.1% formic acid (85:15) Detection: UV 254 nm

Detectors
For narrow peaks, high data rates!!!
Maintaining Resolution at High Analysis Speed



80Hz versus 10Hz (20Hz) Data Rate

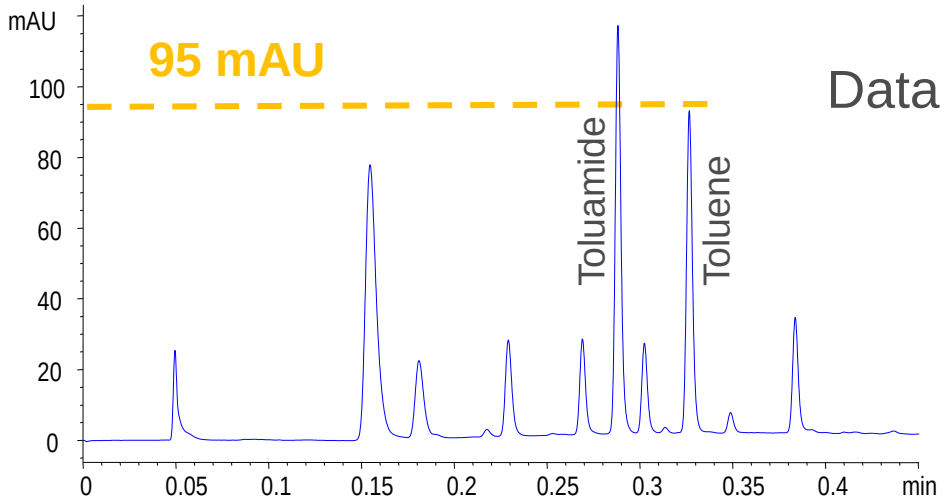
- Peak Width: – 55% (– 30%)
- Resolution: + 90% (+ 30%)
- Peak Capacity: + 120% (+ 40%)
- App. Column Eff.: + 260% (+ 70%)

Data Rate	Peak Width	Resolution	Peak Capacity
80 Hz	0.300	2.25	60
40 Hz	0.329	2.05	55
20 Hz	0.416	1.71	45
10 Hz	0.666	1.17	29
5 Hz	1.236	0.67	16

Sample: Phenones Test Mix
Column: Zorbax SB-C18, 4.6x30, 1.8um
Gradient:: 50-100%ACN in 0.3min
Flow Rate: 5ml/min

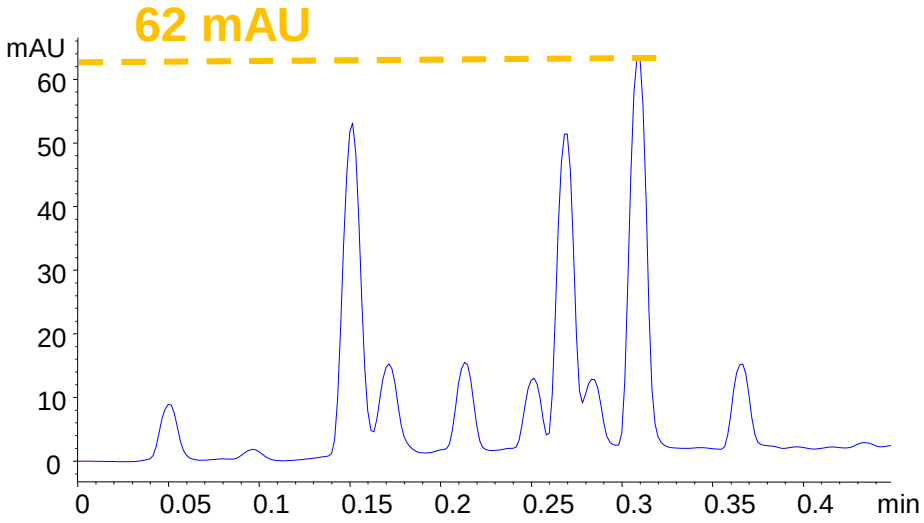
Sensitivity

Data rate – Peak height



Data rate 80 Hz

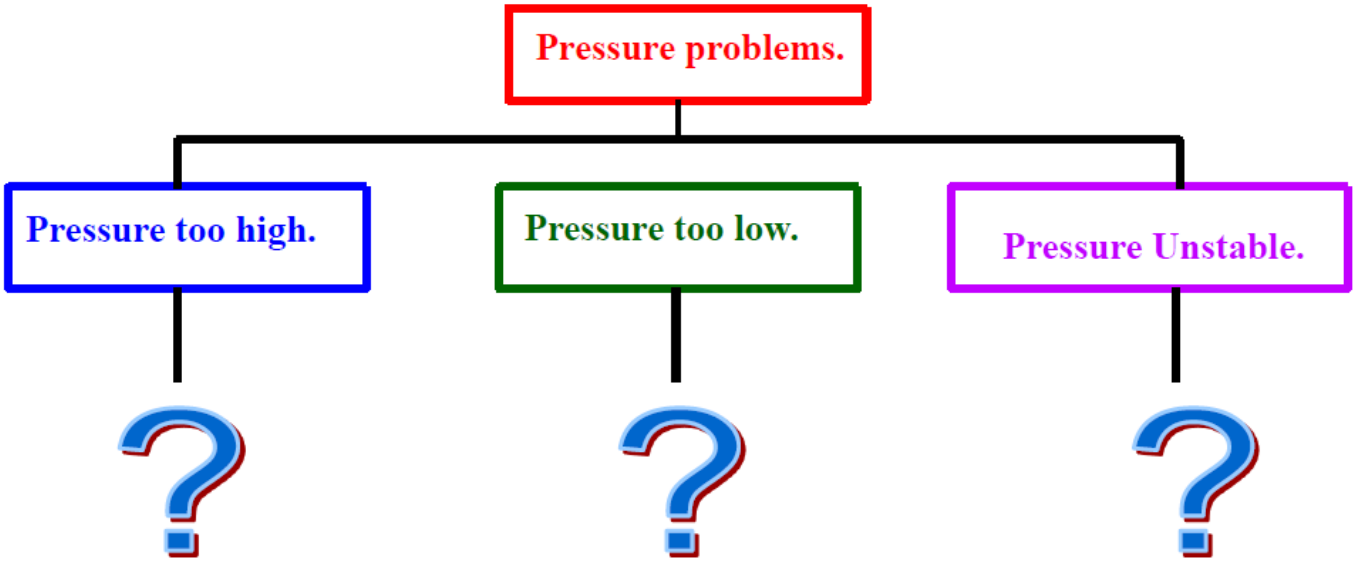
Data rate 10 Hz



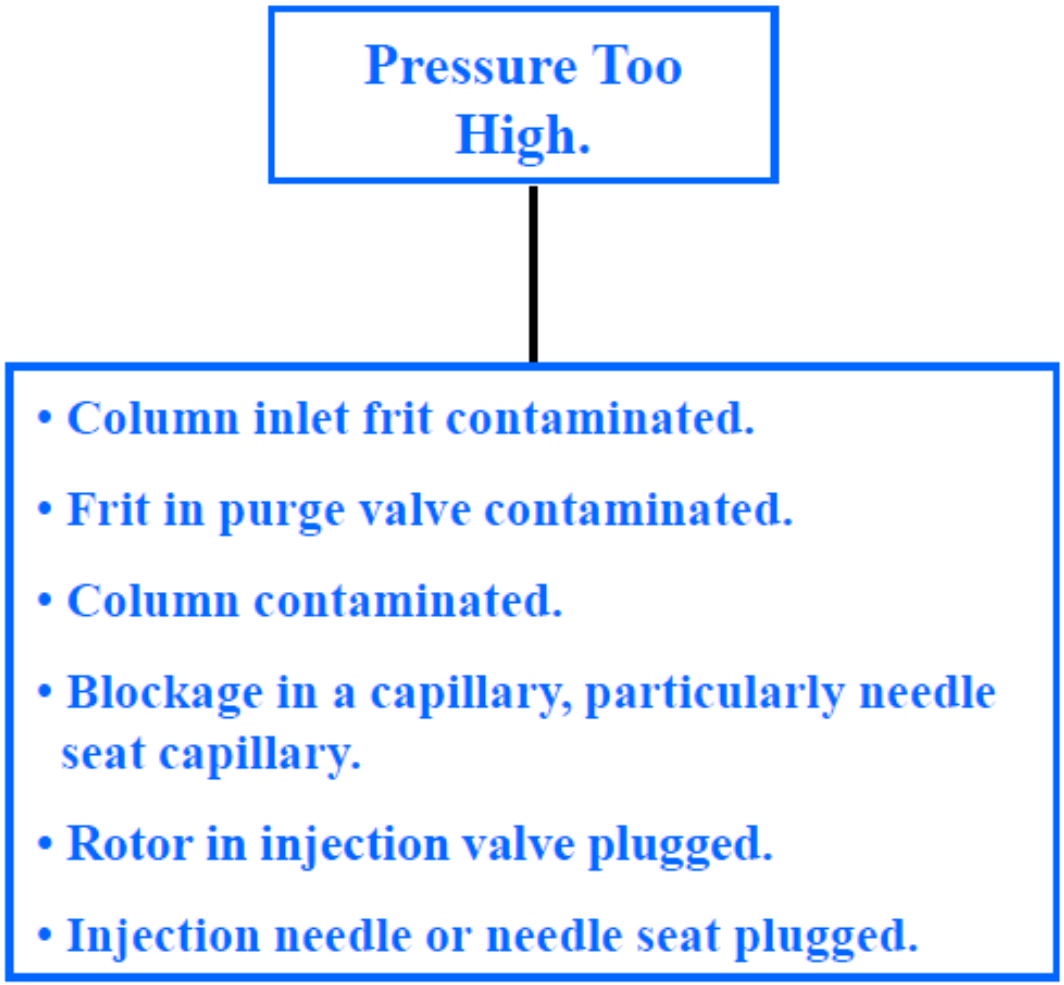
Peak height increases with increasing data rate!

Troubleshooting

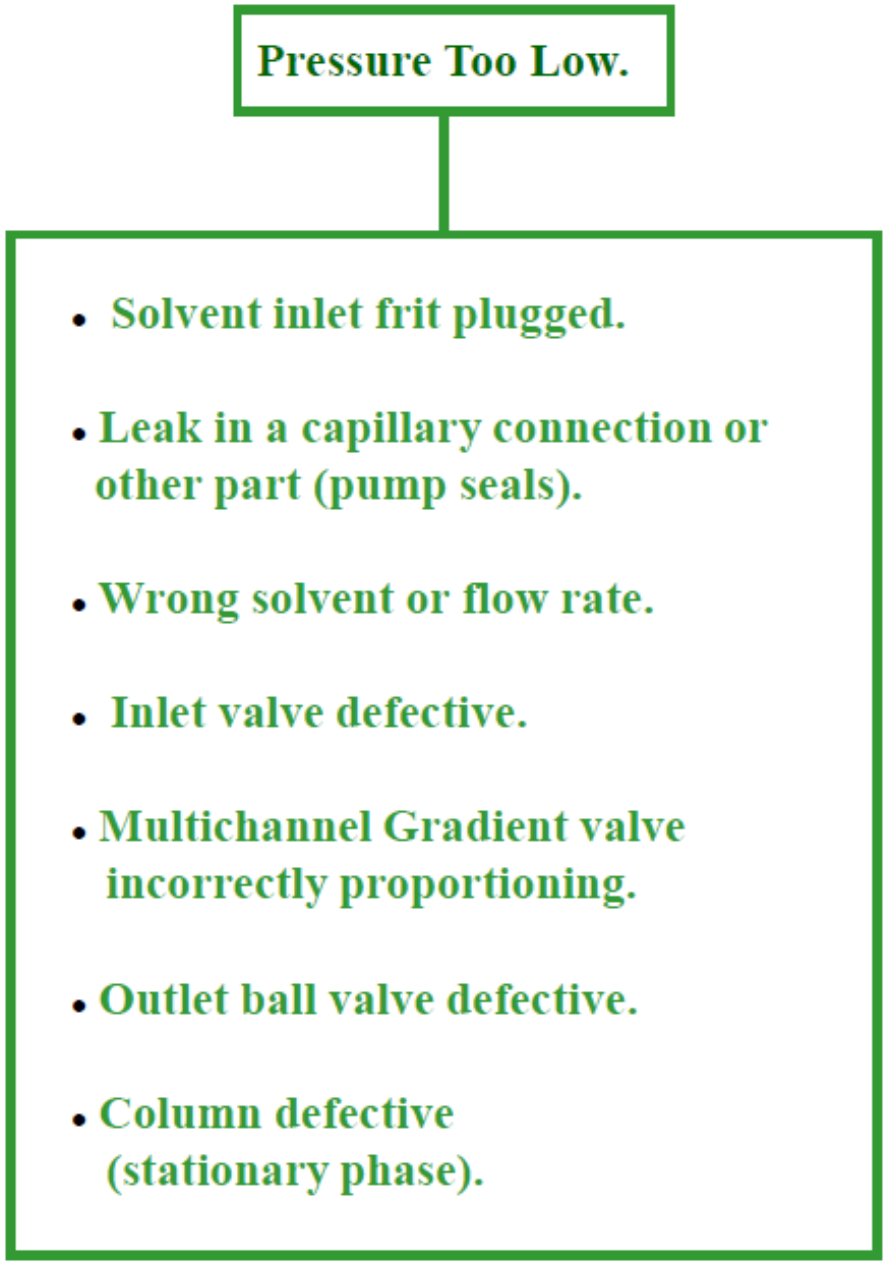
Problems with the System Pressure



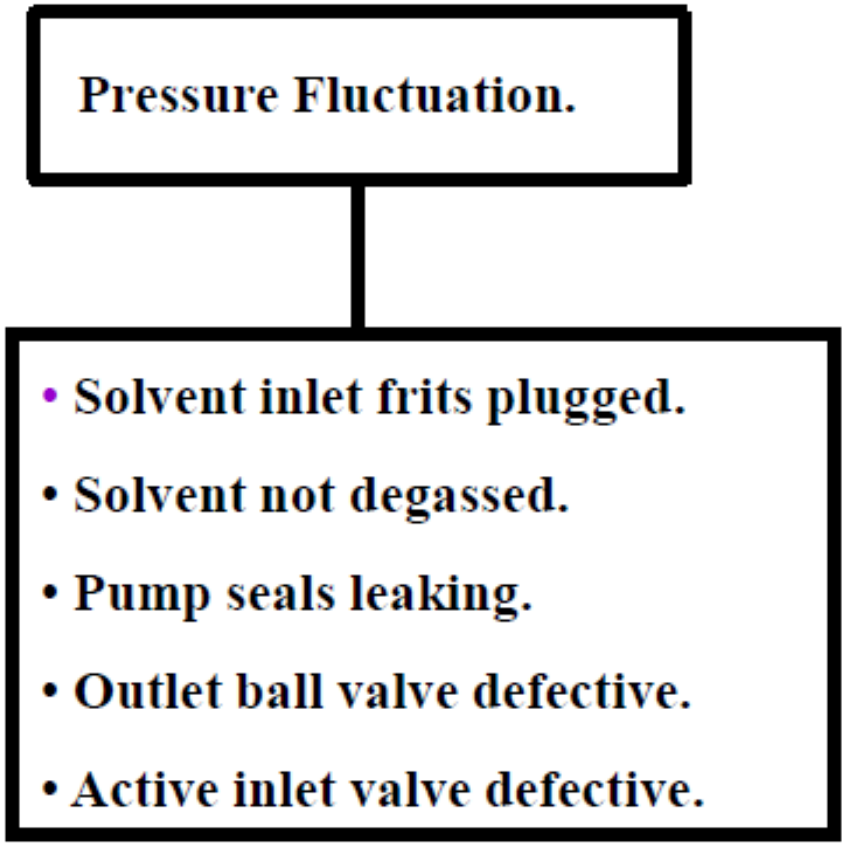
Troubleshooting



Troubleshooting



Troubleshooting

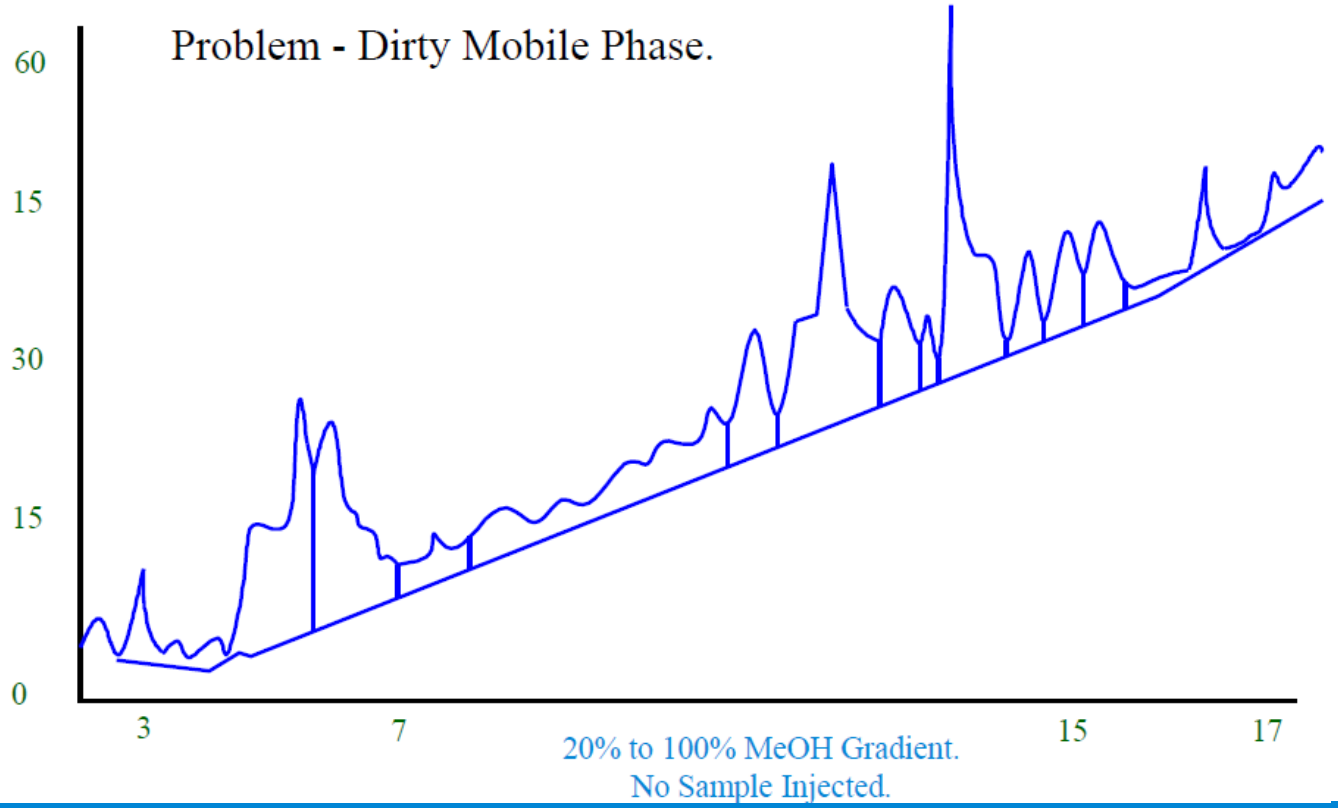


Troubleshooting

Example - Ghost Peaks

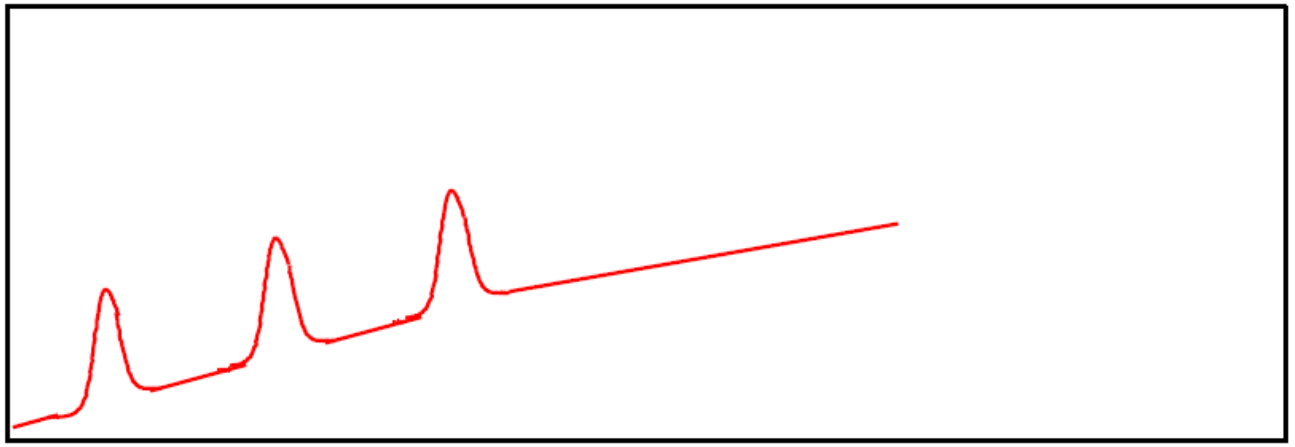
Ghost Peaks - Peaks which appear even on blank injections.

Problem - Dirty Mobile Phase.



Troubleshooting

Drifting Baselines



- Gradient Elution
- Temperature Unstable (Refractive Index Detector)
- Contamination in Mobile Phase
- Mobile Phase Not in Equilibrium with Column
- Contamination Bleed in System

Conclusions

Understanding the HPLC Flow Path and Moving Parts will help you to Keep your System Running as well as Diagnose Problems

Potential symptoms

- High pressure
- Undesirable peak shape
- Changes in retention time/selectivity

Knowing how to properly maintain your system and use good housekeeping can prevent common problems

- Proper purging and changing of solvents
- Cleaning your system
- Timely PMs of the common consumables

Intuvo 9000 GC has a Flexible Compatible Design

Configurable to any application

- SSL, MMI, GSV, LSV inlets
- FID, TCD, ECD, NPD, FPD, NCD, SCD detectors
- SQ and TQ mass spectrometers
- Headspace, thermal desorption, purge and trap samplers
- 16-, 50-, 150-position auto-injectors and trays
- Acquisition Software: OpenLAB and MassHunter
- DA Software: OpenLAB, MassHunter, and ChemStation

Innovating a New Path to GC Productivity

A whole new way to GC

Disposable
Guard chip

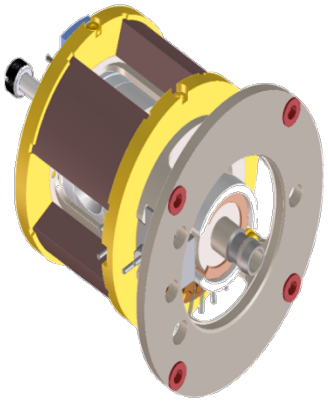
Ferrule-free click-and-run
connections

No-trim
column

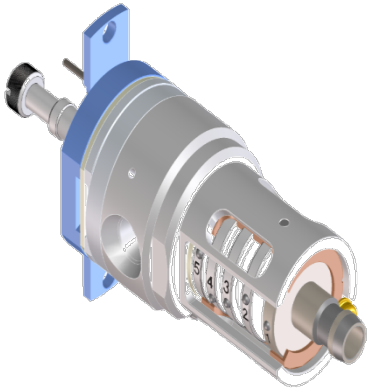
Direct
heating

Modular
Intuvo flow chips

Novel and Proprietary EI GC/MS Source Design



NEW! 7010 High Efficiency Source



7010 High Efficiency Source with Magnet Removed

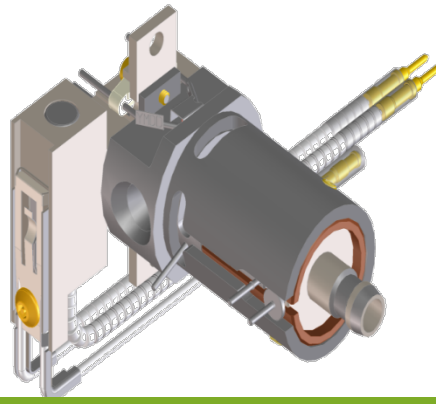
More intense electron beam

X

Longer path length for electron beam/effluent interaction

=

Up to 20x More Ions Produced



7000C Extractor Source

New High-Efficiency EI source

- Features a completely new EI source design – produces 20-30X more ions
- 7010 Instrument Detection Limit (IDL):
  0.5 fg OFN
  8x better than 7000C
- 5977 HES IDL:
  1.5 fg OFN
  6x better than 5977A
- These specifications must be demonstrated at checkout!