Agilent LC/MS – Care and Feeding

Agilent HPLC – Tips and Tricks

Contamination – many sources

Solvent reservoirs

Solvents

Degasser

Pump

Injector

Column

Sample

YOU – pipette tips, gloves, kim wipes, glassware

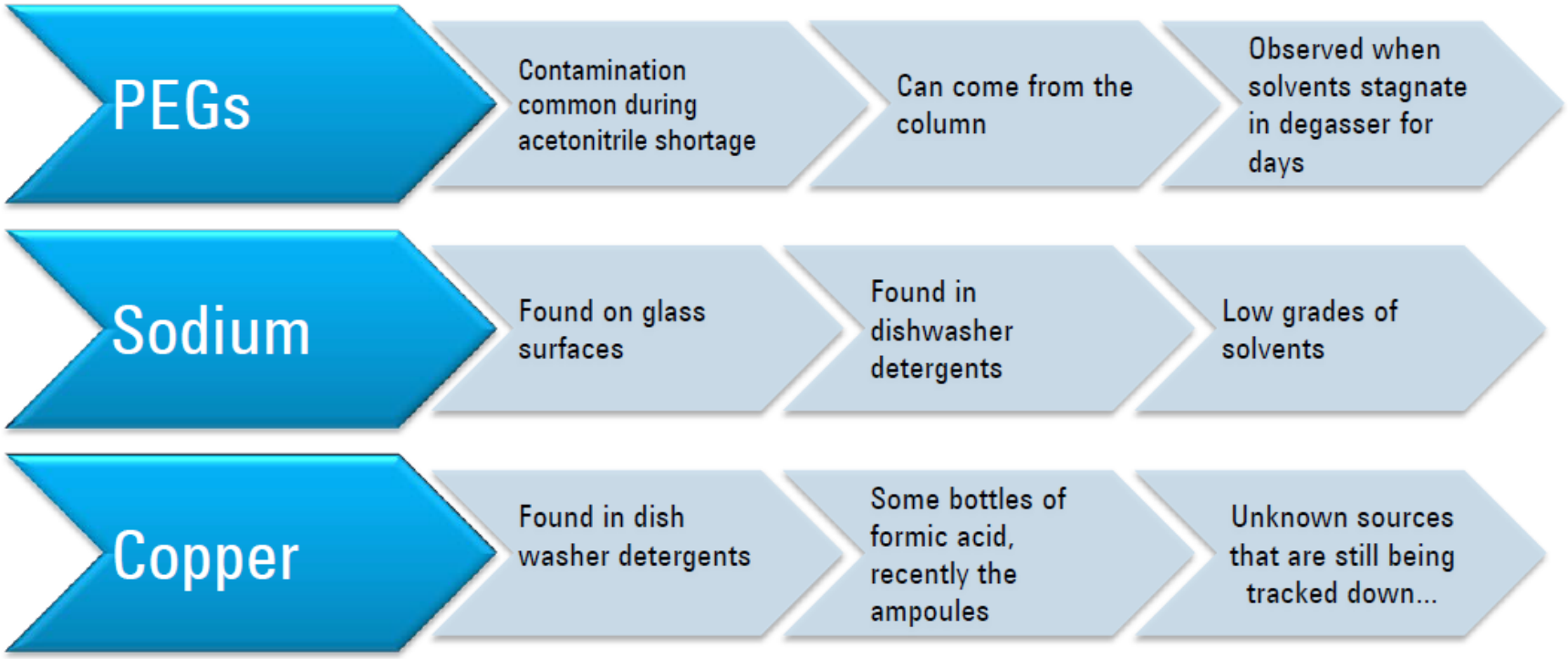
But Rarely the MS

Contamination – What do I do now?

Once the HPLC/MS is contaminated it is important to troublshoot to find the source, but in the end you will need to do a full clean (see appendix).

With a clean system it is a good idea to let the pumps run continuously. Overnight flow of 50uL/min consumes 3mL/hour of solvent. 30/70 A/B with column

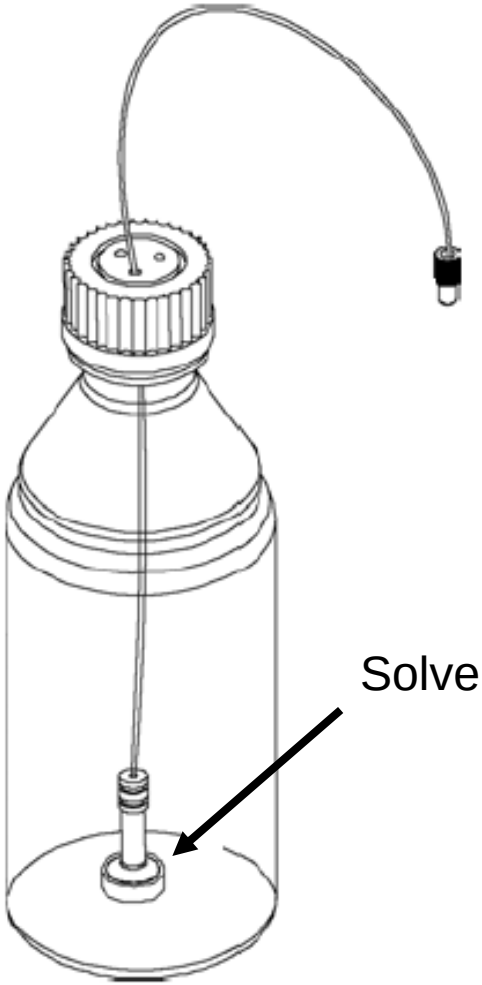
Contamination – many sources



Other Sources of Contamination

- ✓ Using HPLC grade solvents and reagents
- ✓ Filtering the solvents can introduce contamination. It depends on the filter used.
- ✓ Using ParaFilm© as a bottle cap or covering.
- ✓ Gloves, pipette tips, PPT or PET bottles...
- ✓ Use of "Ultrapure water" from a poorly maintained water still
- ✓ Not preventing microbial growth by using water that is 1 week old
- ✓ Phthalate levels even on clean glass surfaces tend to increase with time by airborne adsorption.
- ✓ Storing aqueous mobile phases in non borosilicate glass reservoirs (Note: Shipping solvent bottles from vendors are not borosilicate glass!)
- ✓ Topping off solvents
- ✓ Perfumes, floor cleaning agents, air fresheners, etc.

Solvent Vessels: The number one culprit

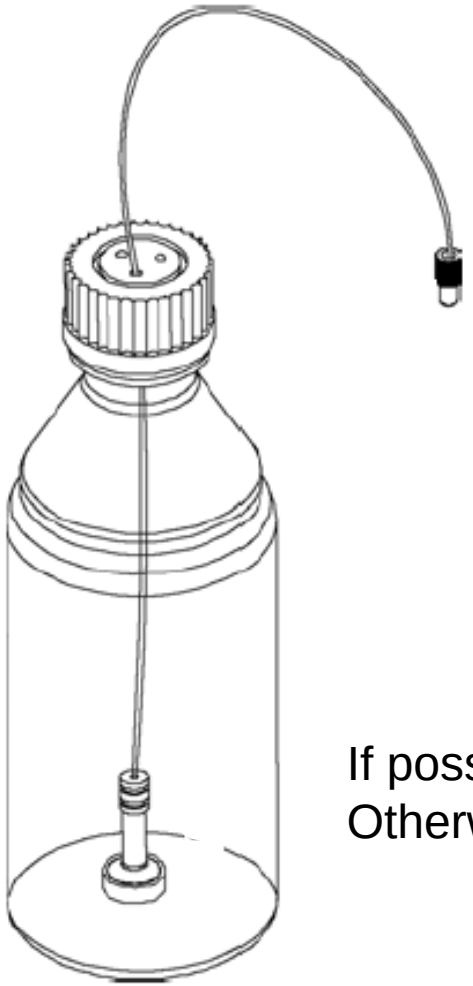


Solvent inlet filter (20 μm).

1. Change reservoirs weekly.
2. DO NOT Top-off
3. Replace the glass filters with Stainless Steel Frits (PN 5022-2192)
4. NEVER use detergents
5. To clean rinse with IPA, MeOH, H2O, MeOH.

Note: Do not sonicate the glass filters.

What‘s in the Solvent Vessels: The number two culprit



1. Contaminated solvents will contaminate the solvent reservoirs
2. Always use Mass Spec grade water (preferably 18mOHM)
3. Buy the smallest volume of formic acid and other modifiers.
4. Always use Mass Spec grade ACN and MeOH

If possible Bake glassware at 400oC for 6 hours
Otherwise: Water then IPA or MeOH then ACN then MeOH

Be Careful!

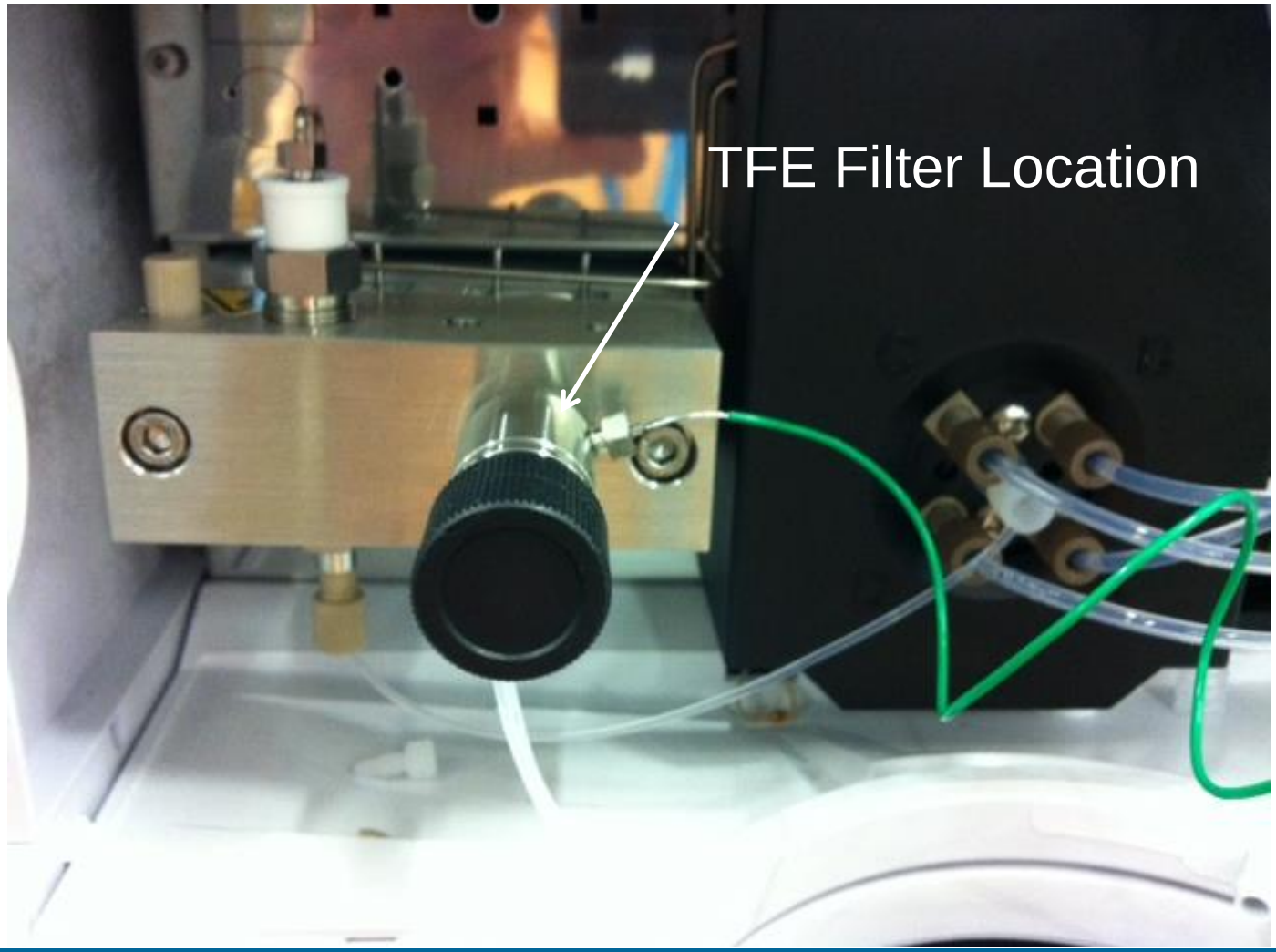
Unless all cleaning steps are followed the system can appear to be in worse condition than before.

High spectral background is not necessarily an indication that a problem exists and a clean background does not guarantee that a problem does not exist.

Changing ANY tubing that has not previously been cleaned and deactivated can make it appear as the entire system is contaminated and will contaminate all components down stream of the change

All garbage will collect on the nearest frit

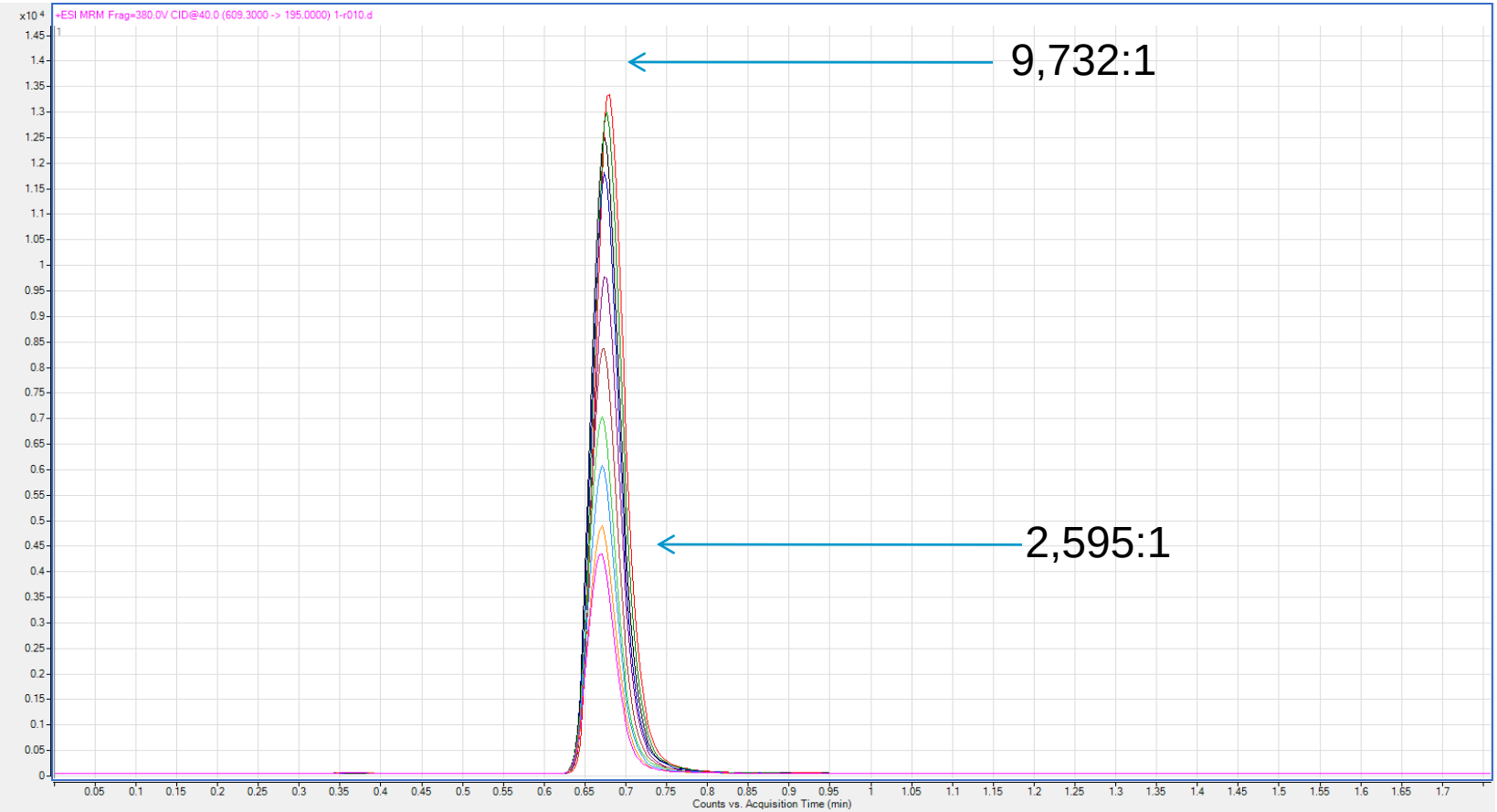
Location of filters in 1260 Infinity Pump



PTFE Frits used in pumps

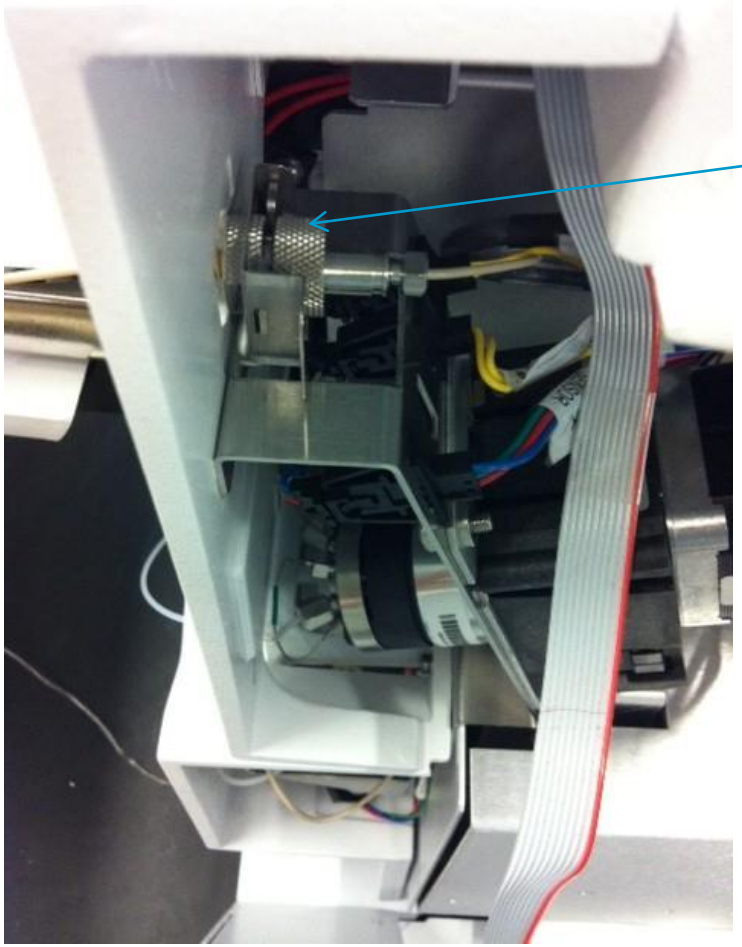
PN 01018-22707

Behavior of dirty PTFE pump frits - QqQ 6490 1 pg Reserpine isocratic check out



The system was “regenerated” at 100% Aq for 2 hours,
S/N decreased 5 fold in ~1 hour

Location of SS Frit in MS



SS Frits used at inlet of all MS

PN 0100-2051

Maintenance Overview

Solvent inlet

Clean or Exchange:

Solvent inlet filter.

Column Compartment

Replace the rotor seal (Column valve).

Check:

Leak sensor.

Drain tube.

Pump

Exchange:

PTFE frit.

Pump seals.

Outlet Ball Valve.

AIV Cartridge.

Wash seals.

Clean:

Pistons.

Support ring.

Check:

Piston springs.

Leak Sensor.

Drain tube.

Autosampler

Exchange:

*Needle.

*Needle seat.

Rotor seal.

Check:

Leak Sensor.

Drain tube.

* ... If necessary.

Detector

Exchange:

*Lamp.

*Cell window.

Clean:

Flow Cell.

Check:

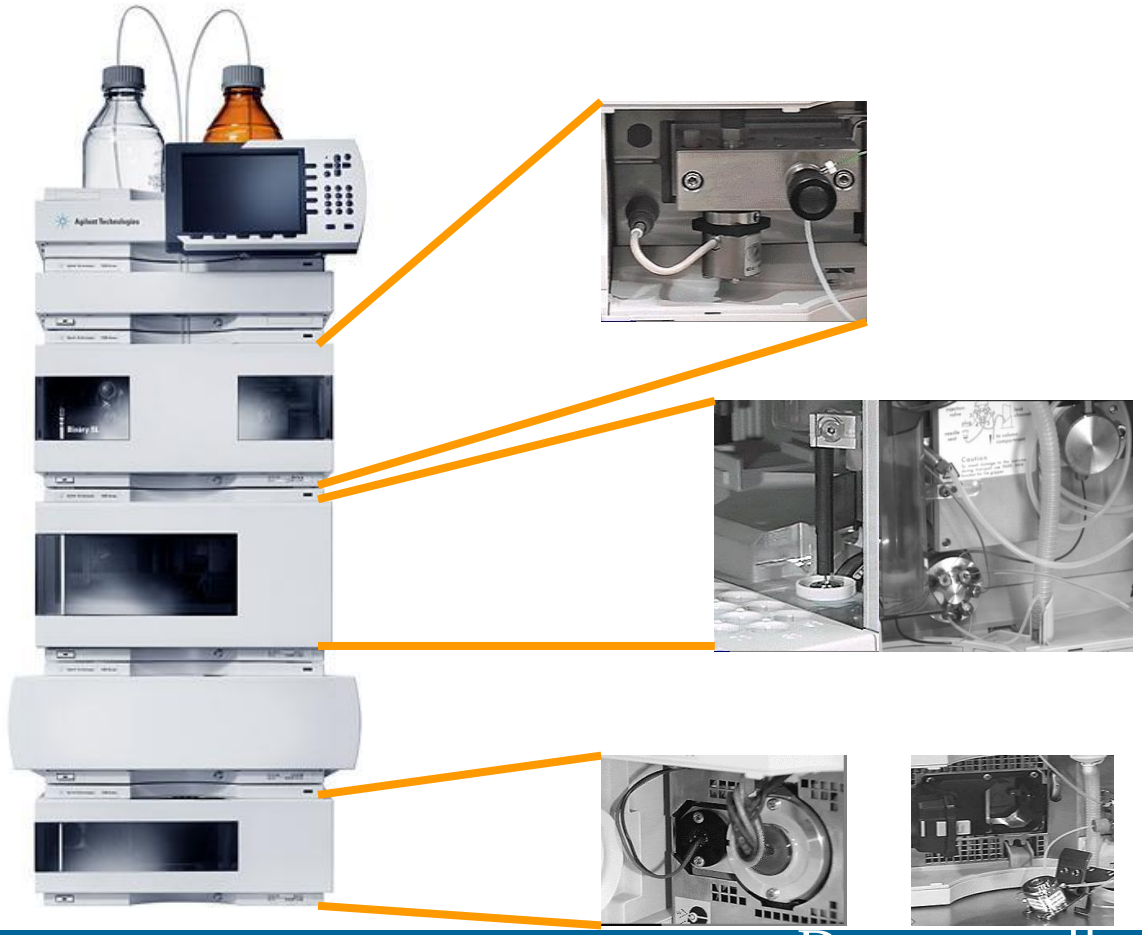
Leak sensor.

Drain tube.

Tests.

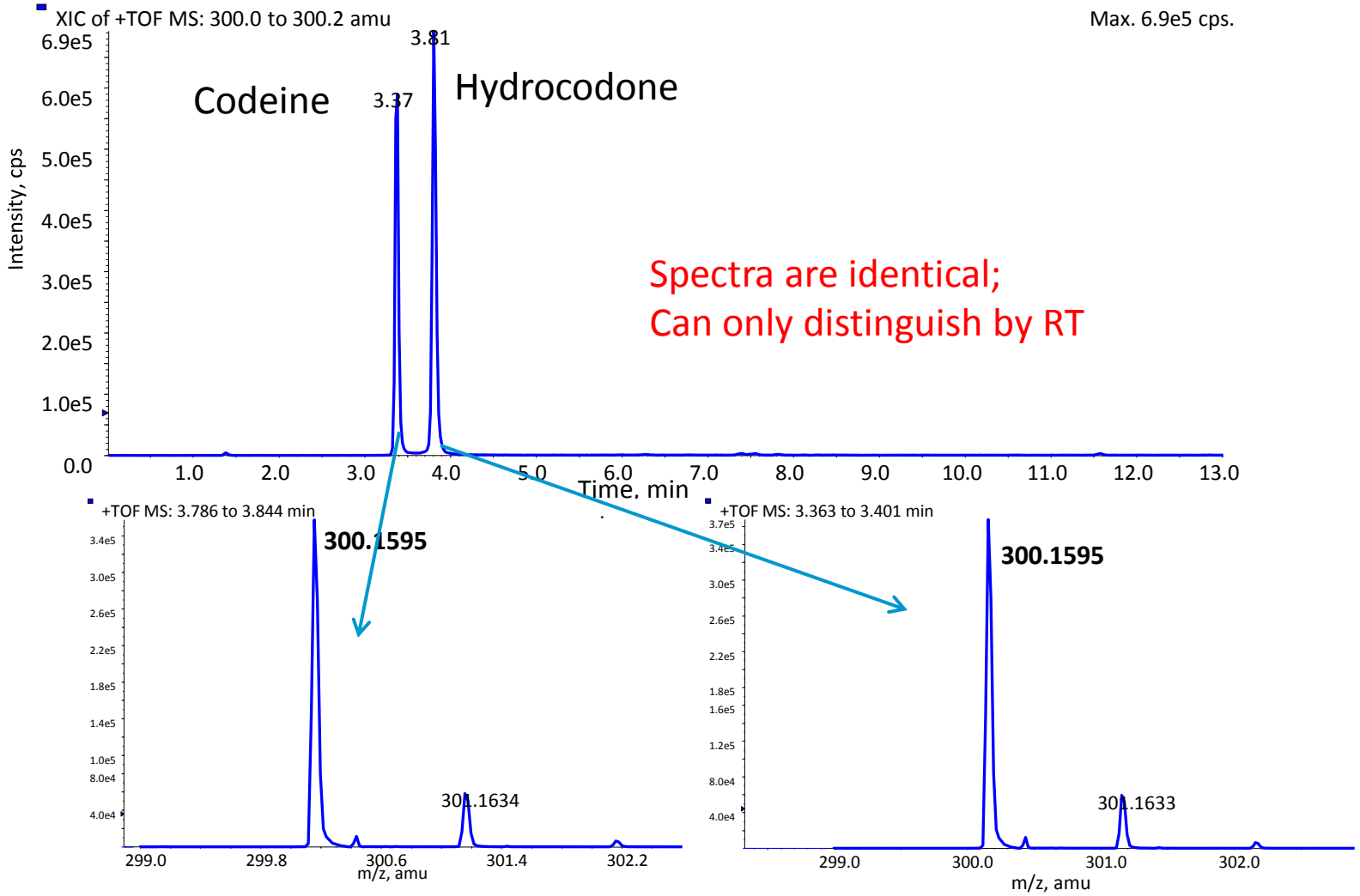
Maintenance Areas of the Agilent 1200 HPLC System

http://www.chem.agilent.com/en-US/search/library/Pages/VideoLibrary.aspx



Detector cell

Chromatography still matters: Identification of isobaric compounds $C_{18}H_{21}NO_3$



HPLC – Tips and Tricks for adapting to MS

Adapting Existing LC Methods to LC/API-MS

Replace non volatile buffers with volatile buffers at a concentration of <10 mM for ESI or <100 mM for APCI

- Substitute phosphates and borates with ammonium acetate, ammonium formate, TFA
- If a non-volatile buffer must be used, select a buffer with only the anionic or cationic part as non-volatile (i.e. ammonium phosphate and keep concentration very low) keeping the column id and flow rate low (2.1 or 1.0 mm id)

Keep the pH the same as in the original separation with volatile additives – formic acid, acetic acid, TFA, ammonium hydroxide

Use volatile ion-pair reagents only when needed – heptaflurobutyric acid (HFBA) and tributylamine (TBA)

Buffers for Electrospray and APCI

Positive Ion (use pH <7.0; 5 preferred)

- Acetic acid, CH3COOH
- Acetic acid with 5-10 mM Ammonium Acetate
- Formic acid, HCOOH
- Formic acid with 5-10 mM Ammonium Formate
- Trifluoroacetic acid, CF3COOH (typically use 0.1% or less)

Negative Ion (pH > 7.0; 9 preferred) – make sure HPLC column is compatible

- Ammonium hydroxide
- Triethylamine (Creates a persistent positive ion at 102 m/z
- Other trialkylamines (greater persistent background in positive mode)

Post-column addition of acid or base may be used to adjust the pH if the chromatography won't work at the desired pH

Considerations for LC Performance with MS

Helpful Hints for Sample Preparation

Sample Clean-up Methods – eliminating matrix/salt/detergent effects:

- ultrafiltration
- solvent extraction/desalting
- liquid-liquid extraction
- solid phase extraction (SPE)
- immunoaffinity
- on-column concentration
- column switching (LC/LC)

If salts and detergents cannot be avoided, remove them using chromatography (short column is sufficient) or cut-off filter

Concentration issues –Dilute sample in LC method starting conditions

Sample Matrix Effects

The MS hardware is robust and tolerates non-volatile components

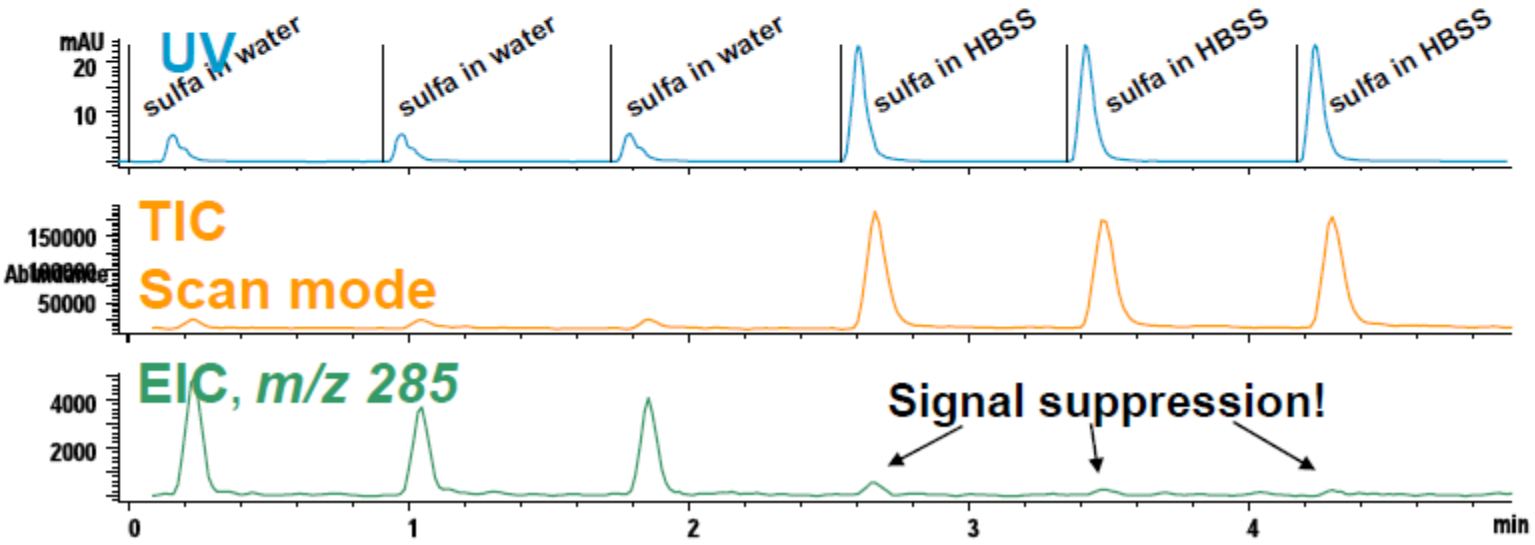
however...

The ionization process is effected by the concentration and type of salt/buffer

Sample Matrix Effect

Sulfachloropyridazine dissolved in water vs. Hanks Balanced Salt Solution (HBSS)

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



LC/MS Methods Summary

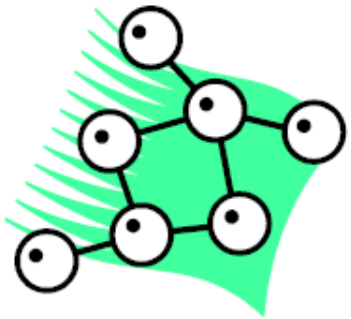
For analytes that ionize in solution

- Adjust pH to promote ionization
- Use AP-ESI with volatile buffers <25 mM
- Lower flows and higher concentrations

For analytes that will ionize and are VOLATILE

- Solvent is important to chemical ionization (e.g. MeOH)
- Use APCI with volatile buffers <100 mM
- Higher flows and more analyte

Watch for Matrix effects



~~Magic~~ Mass Spectrometry (but really m/z Spectrometry)

Benefits of LC/MS

For the chromatographer

- Complements existing LC detectors
- Does not depend on particular functional group
- Can be used as a mass-specific detector
- Provides both qualitative and quantitative information

For the mass spectrometrist

- Can analyze compounds not amenable to GC (large, polar, thermally labile)
- Allows direct coupling of LC separation; produces better information faster than "offline" LC/MS

Interfacing HPLC To MS

HPLC

High pressure liquid phase separation

Produces high gas load

No mass range limitation

Can use inorganic buffers

MS

High vacuum required

Tolerates limited gas load

Elevated temperatures

Depends on m/z and analyzer

Prefers volatile buffers

Most common HPLC MSs

- Serial Measurement Systems – e.g. Quads/Traps
  - Each m/z passed through the MS analyzer one at a time
  - High sensitivity for selected ions
  - Predominant use: Quantification of known compounds
- Parallel Measurement Systems- e.g. TOF's
  - Multiple m/z passed simultaneously
  - Sensitivity over broad mass range
  - Predominant use: Identification using mass spectra

Common HPLC MS Types

- Single Quadrupole (quad, MS, single quad…)
- Triple Quadrupole (triple quad, QQQ,…)
- Time of Flight (ToF…)
- Quadrupole Time of Flight (QToF…)
- Ion Trap (trap, linear trap, 3D trap, Orbitrap…)

Single Quadrupole

Strengths

- Robust, simple operation
- Tolerant of non-volatile salts and background ions
- Low cost
- Lower scan speed
- High sensitivity for quantification in SIM mode

Limitations

- Lack of structural (MS/MS) info
- Lack of exact mass information
- Lower m/z range
- Slower “scan” speed than (Q)TOF

Triple Quadrupole

Strengths

- Best sensitivity (S/N) for quantitative studies in complex matrices (MRM)
- Multiple fragmentation produces more product ions
- Neutral loss and precursor scan
- Rugged operation

Limitations

- Scan speed slower than Ion Trap, TOF and SQ
- Lower m/z range
- MS/MS info but no MS^n

Ion Trap

Strengths

- **Multiple stage MS (MS^n)**
- **Fast scanning (up to 26,000 Th/s)**
- **Lower Resolution** (depends on scan speed)
- **Full scan MS/MS with higher sensitivity than QQQ**
- **Auto generation of MS/MS**

Limitations

- **No exact mass information (0.2Da)**
- **No true quadrupole SIM - less sensitive vs. SQ/QQQ for low level quant studies**
- **Less fragmentation than QQQ in MS/MS**
- **Lower m/z range**
- **Low mass cut-off (~1/3 of precursor mass)**
- **Smaller linear dynamic range**

TOF

Strengths

- Accurate mass
- High resolution
- Fast sampling rates
- Excellent “scan” sensitivity over a wide mass range
- Very high m/z range

Limitations

- Restricted MS/MS capability
- Dynamic range limited for some quantitative studies

QTOF

Strengths

- **Combination of MS/MS with accurate mass information**
- **High resolution**
- **Fast sampling rate**
- **High specificity**
- **Excellent "scan" sensitivity over a wide mass range**
- **Very high m/z range**

Limitations

- **Quantitative analysis (dynamic range and sensitivity)**

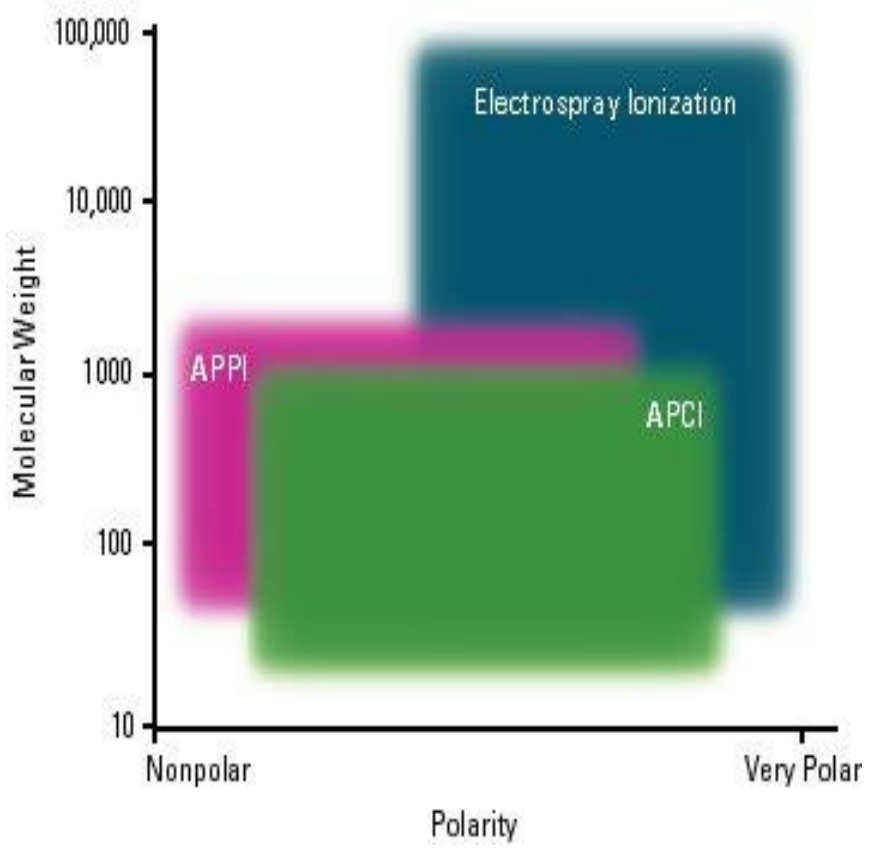
Considerations For Coupling MS To HPLC

Realizing The Importance Of Ionization

- MS detection requires the formation of ions
- Consistent, stable ion formation is the foundation for reproducible measurements
- Ion formation is driven by well understood chemistry. It is **NOT** magic
- Ion formation in the liquid to gas interface (source) is the key to success.
- The rest of the MS is a robust instrument requiring little attention for routine analyses

When to use which source

- **Ions form by several but distinct mechanisms**
- **AP-ESI works for ions that form in solution**
- **APCI works for volatile compounds that form ions in gas phase**



General Comparison – ESI vs APCI

ESI

Ionization: Pre-formed analyte ions transferred to gas phase

Mobile Phase Issues:

- Organic Solvent:

  little effect on ionization

- pH: key to pre-formed ions

- Buffer Concentration: < 25 mM

- Flow Rate: < 0.5 ml/min

APCI

Ionization: Charge exchange of gas phase neutral analytes

Mobile Phase Issues:

- Organic Solvent:

  MeOH usually best

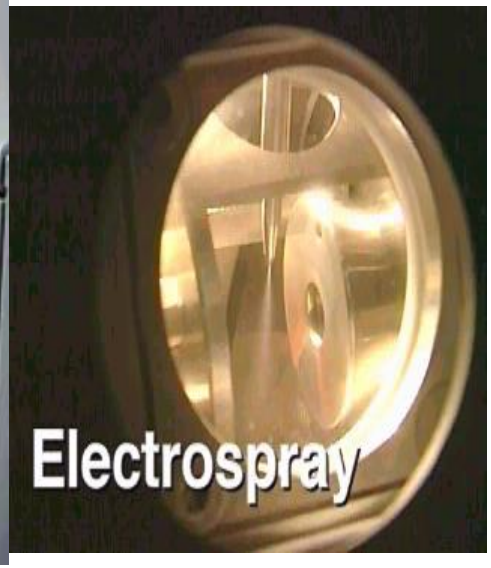
- pH: neutral analytes

- Buffer Concentration: < 100 mM

- Flow Rate: > 0.5 ml/min

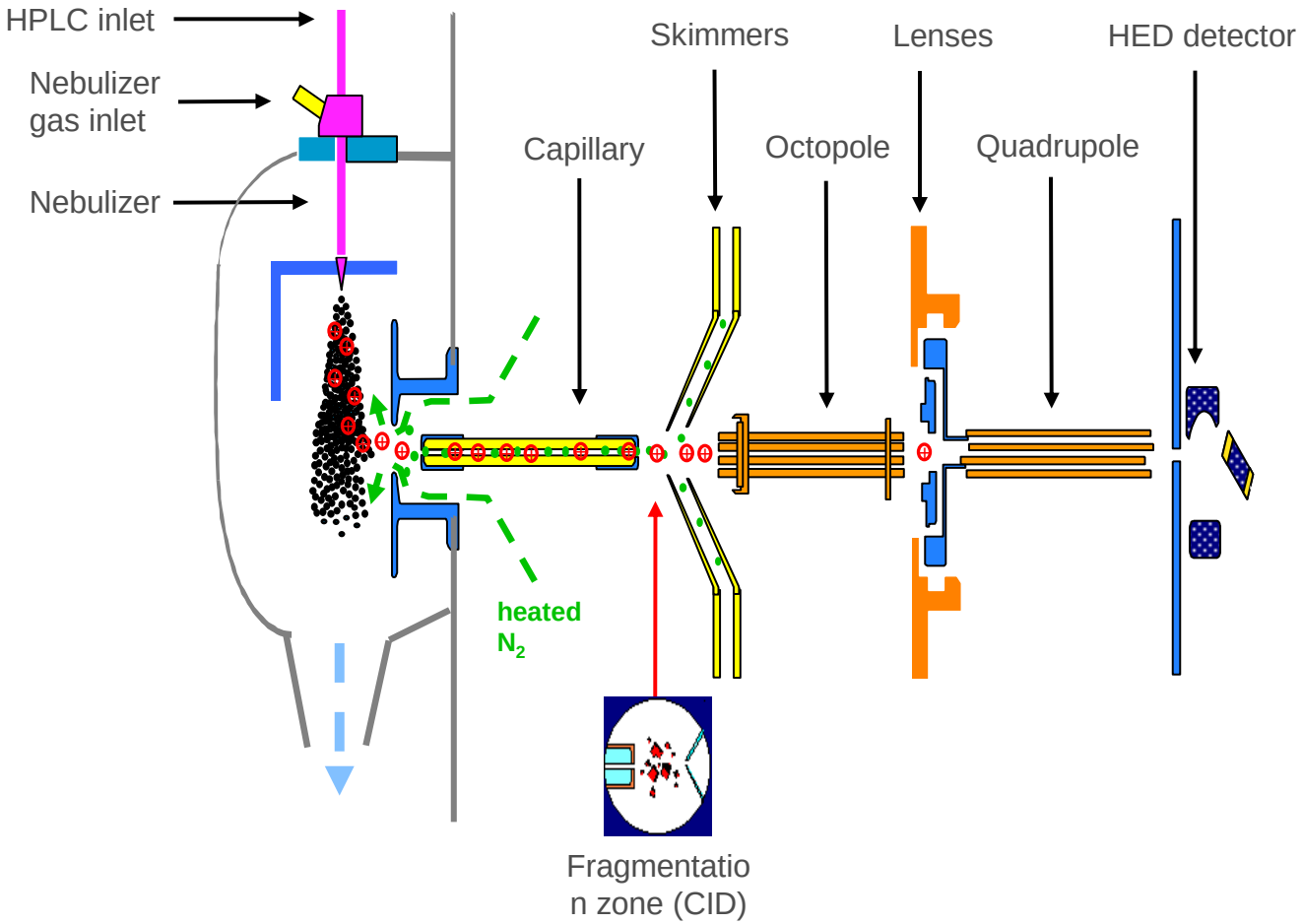
Orthogonal Spraying

- Enhanced sensitivity
- Buffer/matrix tolerant
- Broader flow rate range
- Optimized geometry
- Higher signal-to-noise
- Reduced downtime/maintenance

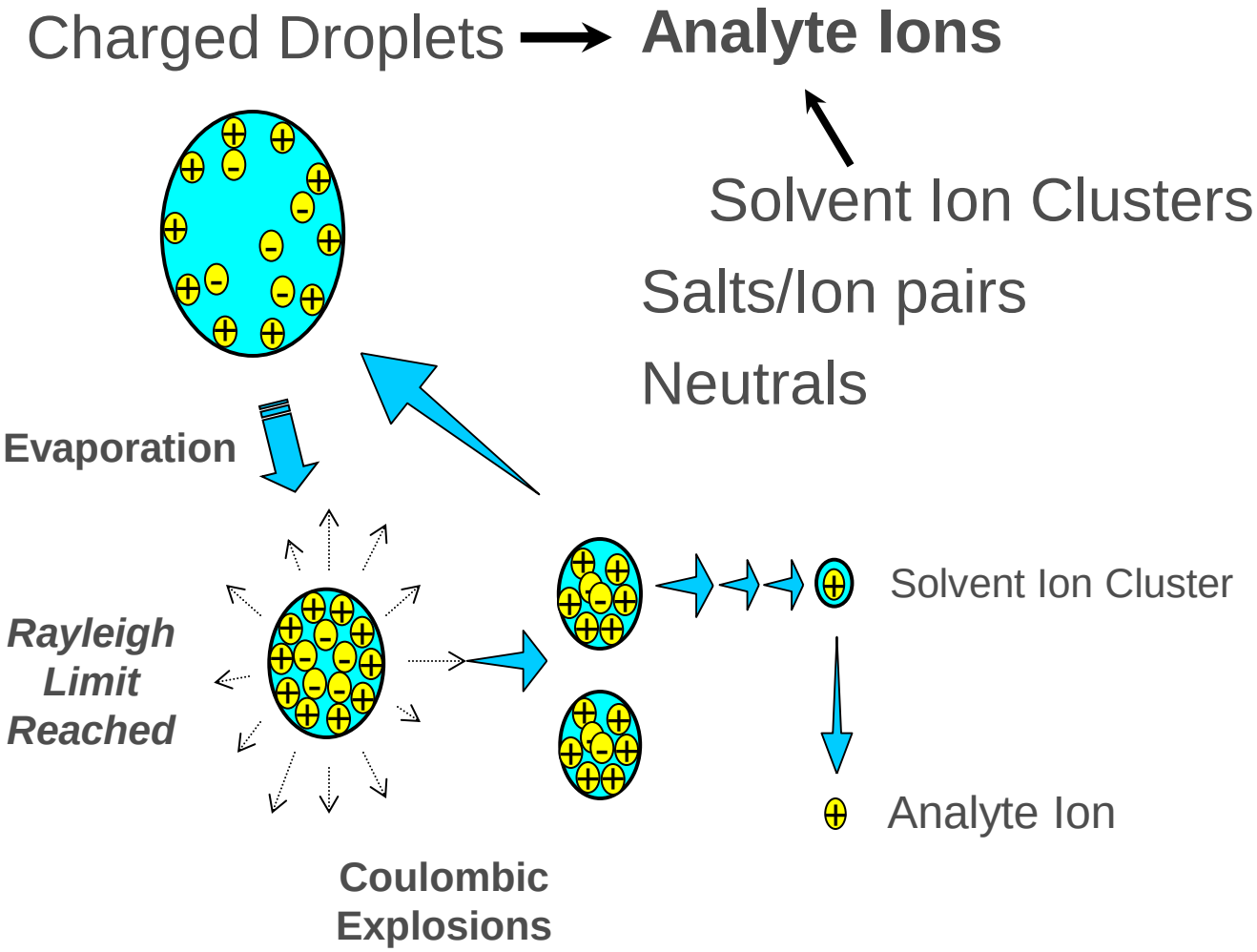


Electrospray

Spray into a high electric field and evaporate



Electrospray Theory



Electrospray LC/MS

Advantages

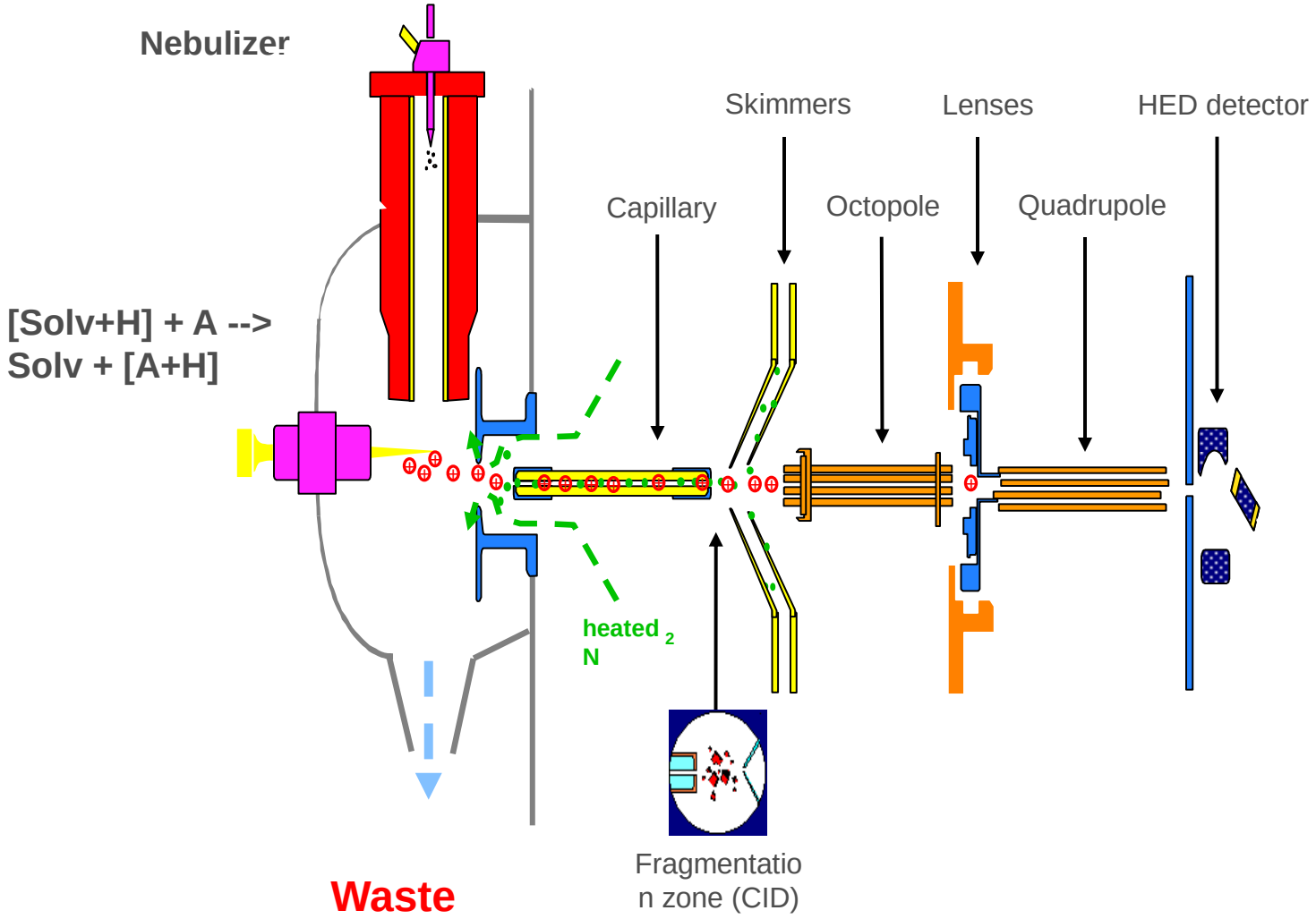
- Softest ionization available
- LC/MS interface with best sensitivity (if analyte amendable)
- Extends mass range for multiply charged analytes
- For a wide range of medium to high polarity compounds
- More sensitive at lower flow rates
- Low maintenance

Disadvantages

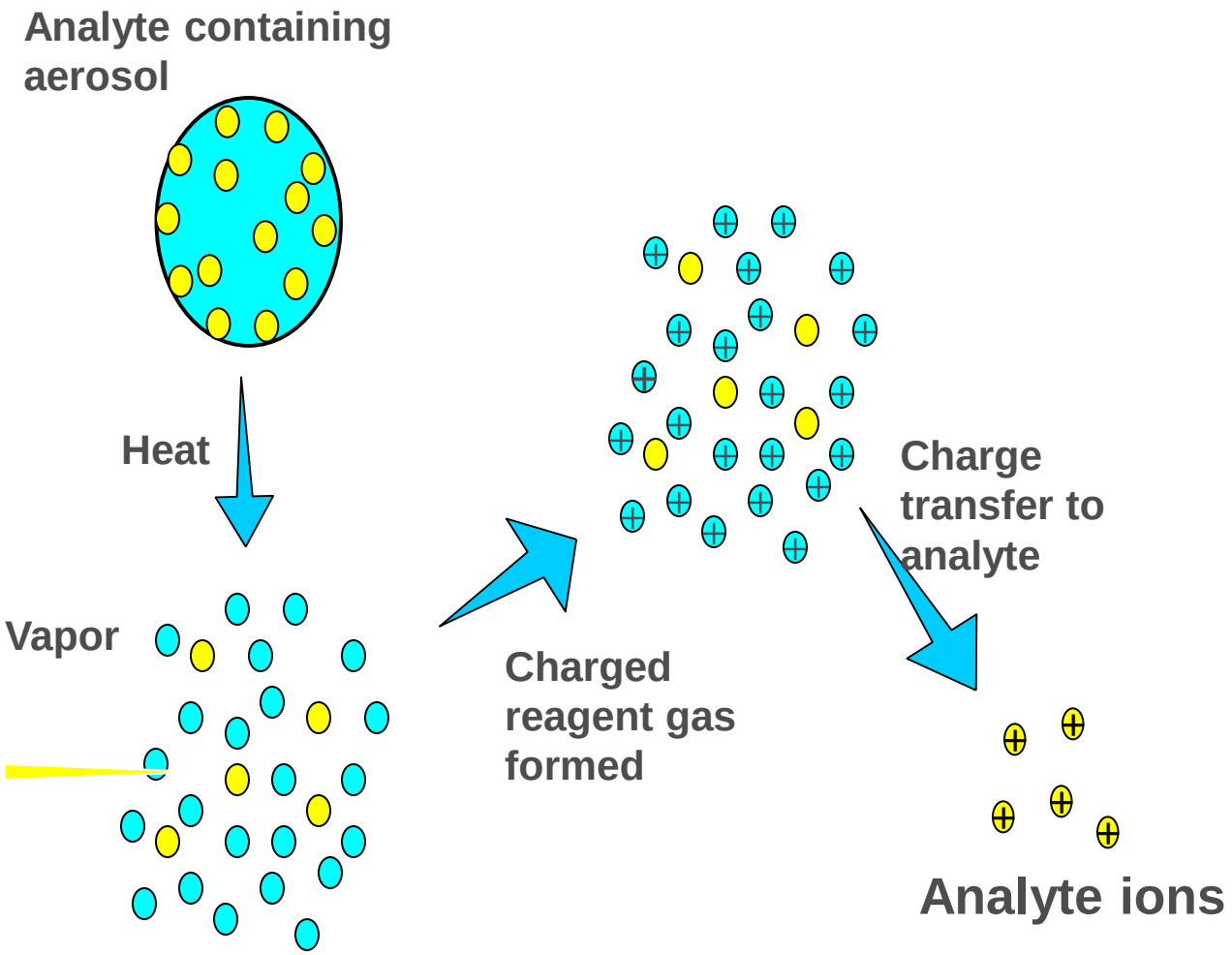
- Solution chemistry influences ionization process
- Adduct ions (other than M+H) possible with some analytes
  - M+Na, M+K, M+NH_4
- More compounds respond thus higher background

APCI – Atmospheric Pressure Chemical Ionization

Vaporize in gas phase and ionize the gas with a discharge



APCI Process



APCI LC/MS

Advantages

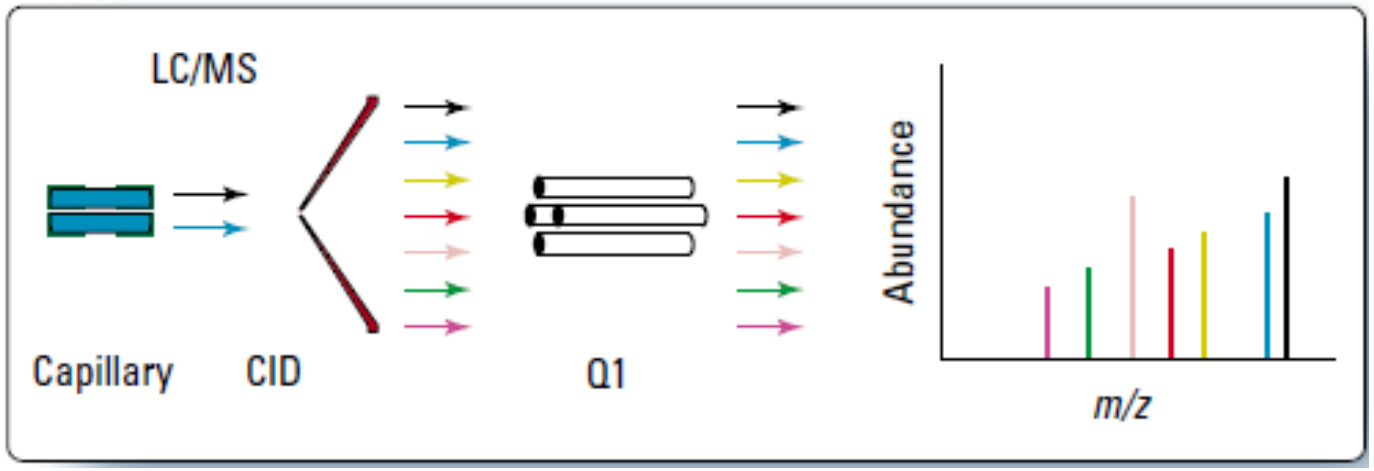
- Less sensitive to solution chemistry effects than electrospray
- Lower background than electrospray.
- Good sensitivity for compounds of intermediate MW and polarity
- Complementary to electrospray for less polar analytes
- Tolerates higher flow rates without decrease in sensitivity

Disadvantages

- Less useful for thermally labile compounds
- Requires some compound volatility

Adding MS Detection to HPLC methods typically does not require many changes. Ways you can optimize your existing HPLC method…….

- Ensure adequate analyte concentration
- Maximize ionization through careful selection of solvents and buffers
- Minimize the presence of compounds that compete for ionization or suppress signal.
- GOOD CHROMOTOGRAPHY COUNTS



Agilent MS – Tuning and Calibration

Terminology

Tuning

- adjustment of the mass spectral peak shape and the resolution between peaks of two adjacent m/z values

Calibration

- mass calibration to assign a digital-to-analog conversion (DAC) value to mass spectral peaks of a substance that produces ions of known m/z values

Tune Masses

Electrospray Tuning Mix (Bottle B)
PN G2421A

Masses	Positive	Negative
*Mass 1	118.08	112.99
Mass 2	322.05	431.98 f
*Mass 3	622.03	601.98
*Mass 4	922.01	1033.99
*Mass 5	1521.97	1633.95
*Mass 6	2121.93	2233.91
Mass 7	2721.89	2833.88

APCI Tuning Mix (Bottle A)
PN G2422A

Masses	Positive	Negative
*Mass 1	121.05	119.04
Mass 2	322.05	
*Mass 3	622.03	556.00
*Mass 4	922.01	805.99
*Mass 5	1521.97	1305.95
*Mass 6	2121.93	1805.92

f = ion appears with fragmentor @ 140 V
*Masses in red are autotune masses for the SL
VL masses are those with m/z < 1500

When to Tune and Calibrate

- **Daily** (when running samples)
- In Tune context, turn on Cal B and verify all tune ions present. (Deselect those not needed.
- Print a Tune report from Tune by doing a **Checktune**.
- Always do Positive ion; do Negative ion if used regularly and at least monthly to verify negative ion performance.

When to Tune and Calibrate

.

Weekly

- Verify resolution and accuracy.
- Run **StandardTune** if **CheckTune** does not give satisfactory results.

When to Tune and Calibrate

 Monthly (suggest first week of each month) or **As Needed** (resolution, peakshape degradation)

- Do **Standard Autotune** to re-optimize ion optics, resolution and mass axis.
- **InitialTune** checks and adjusts detector gain to maintain constant response.
- Current Agilent practice is to rarely use Quicktune, only tunes a subset of resolution parameters.

Maintenance of MS

Daily

- Wipe interior surfaces of ESI spray chamber with clean cloth or ~~Kimwipe~~ and solvent (IPA/water or MeOH/water).
- Rinse spray chamber with solvent (*not* into ion transfer capillary!).
- Wipe off spray shield; check tip of nebulizer (and nozzle for Jetstream source) for buildup of solids.
- For APCI: remove corona needle and wipe off lower face of vaporizer.

Maintenance of MS cont'd

Weekly

- Ballast the rough pump for at least 5 minutes.
- For ESI: remove spray chamber and rinse with IPA/water to clean insulating standoffs; clean standoffs with cotton swab.
- For APCI: clean corona needle and lower face of vaporizer with abrasive cloth furnished with system.
- For either: remove spray shield and clean with abrasive cloth; clean capillary cap with ~~Kimwipe~~ lint free cloth and solvent.
- If surface contamination is visible after solvent cleaning, remove and clean capillary cap with abrasive cloth. Use CAUTION

when replacing to avoid damaging gold spring: push straight on, do not twist.

Maintenance of MS cont’d

Semi-annually (suggest January and July):

- Vent system per instructions in User Guide.
- Replace mechanical vacuum pump oil.
- Replace nitrogen purifier or hydrocarbon trap(s) [BHT-4 for use with nitrogen generators].
- Remove ion transfer capillary and rinse with water and organic solvents. There is a new procedure for resistive capillary systems available from the Agilent website or the Service organization.
- After pumpdown and minimum overnight stabilization, run Initial Autotune, both polarities.

Capillary cleaning procedure

- Dissolve 1g Alconox in 100ml deionized water.
- Place ion transport capillary upright in a 100ml polypropylene graduated cylinder and fill with Alconox solution.
- Sonicate the graduated cylinder with the ion transport capillary in an ultrasonic cleaner for 10 to 15 minutes.
- Rinse the ion transport capillary and graduated cylinder several times with dionized water.
- Fill graduated cylinder with deionized water and sonicate the graduated cylinder with the ion transport capillary for 10 to 15 minutes.
- Install the ion transport capillary in LC/MS Desolvation Assembly

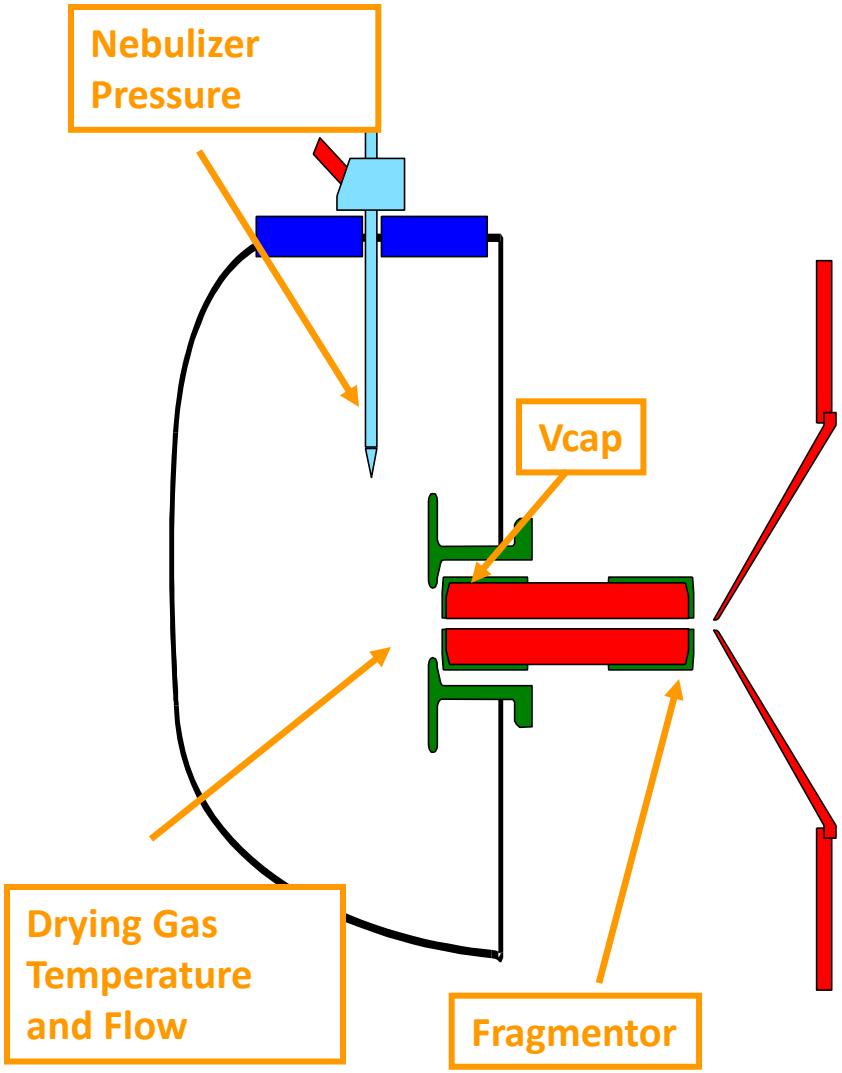
https://www.agilent.com/cs/library/Support/Documents/clng%20glass%20capillaries.pdf

Thank You

Q&A?


Agilent Technologies

Electrospray Spray Chamber Settings



HPLC Flow Rate (µ/min)	Nebulizer Pressure (psi)	Drying Gas Flow (l/min)	Drying Gas Temp (°C)
1 – 10	10 – 15	7	150 - 250
10 – 50	15 – 20	8 - 10	150 - 250
50 – 200	20 – 40	10 - 12	200 - 325
200 – 500	25 – 45	12	325
500 – 1000	50 – 60	12 - 13	350

Drying Gas Flow

- high water needs higher flow
- if too low, spikes in spectra from droplets, dirty cap

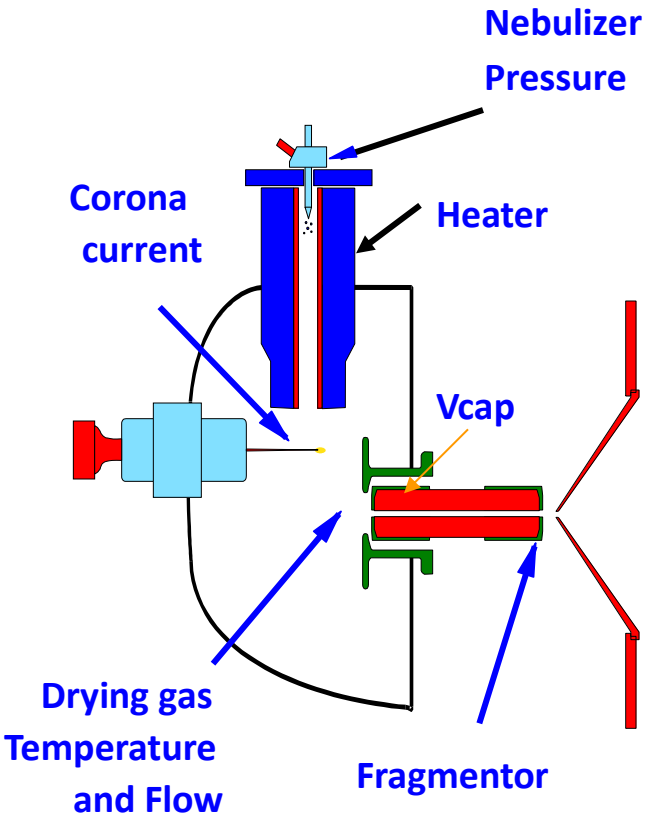
Drying Gas Temperature

- higher for low vapor pressure solvents
- Usually 275 - 350°C

Vcap

- optimize with FIA (2000-6000)
- start with 3000 V
- in negative mode, look for high chamber current or blue glow (indicates corona): reduce Vcap if this happens

APCI Spray Chamber Settings

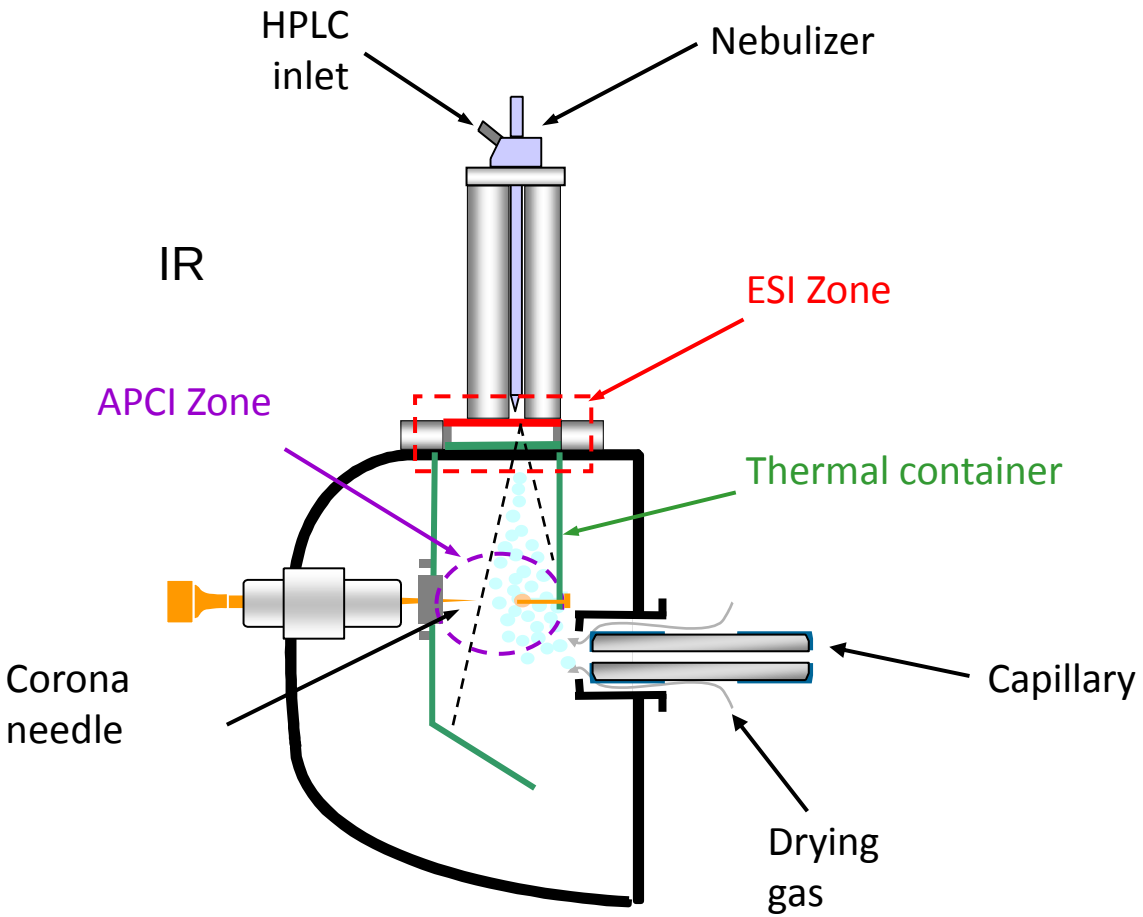


HPLC Flow Rate (µl/min)	Nebulizer Pressure (psi)	Drying Gas Flow (l/min)	Drying Gas Temp (°C)
200 – 500	30-40	5 - 8	300-350
500 - 1000	40-60	7 - 8	350

* Use enough dry gas, but don't remove reagent

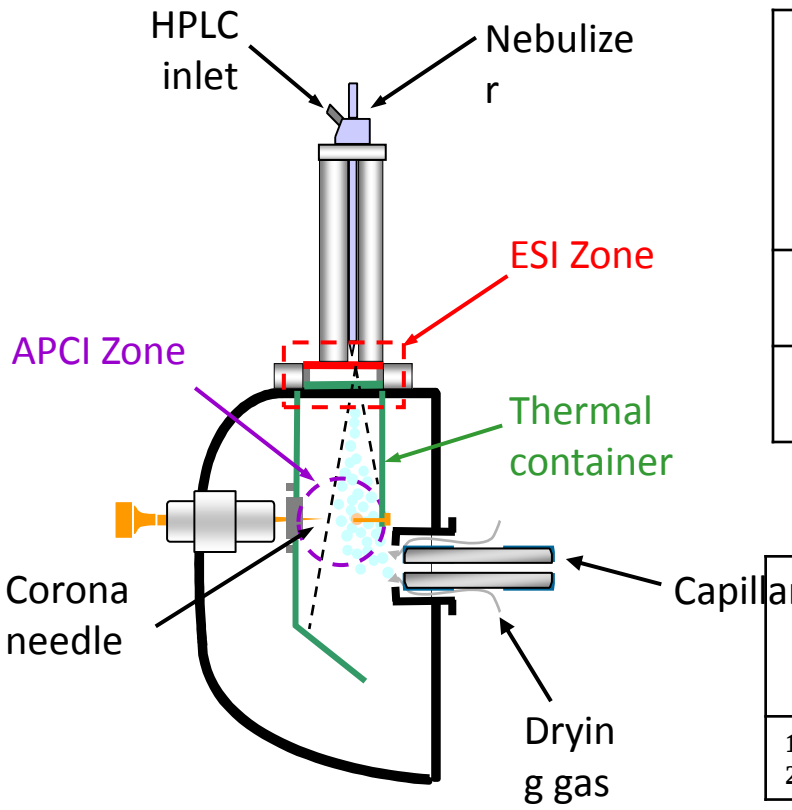
APCI Vap Temp (°C)	Corona Current (nA)	Capillary Voltage (V)
300 – 500	4000 (pos) & 20,000 (neg)	3500

Overview of the Multimode Source



To convert a method developed with any other source, all that is needed is to go to method and run control and change the Method spray chamber to MM-ES+APCI. Spray chamber values will be the same as for APCI

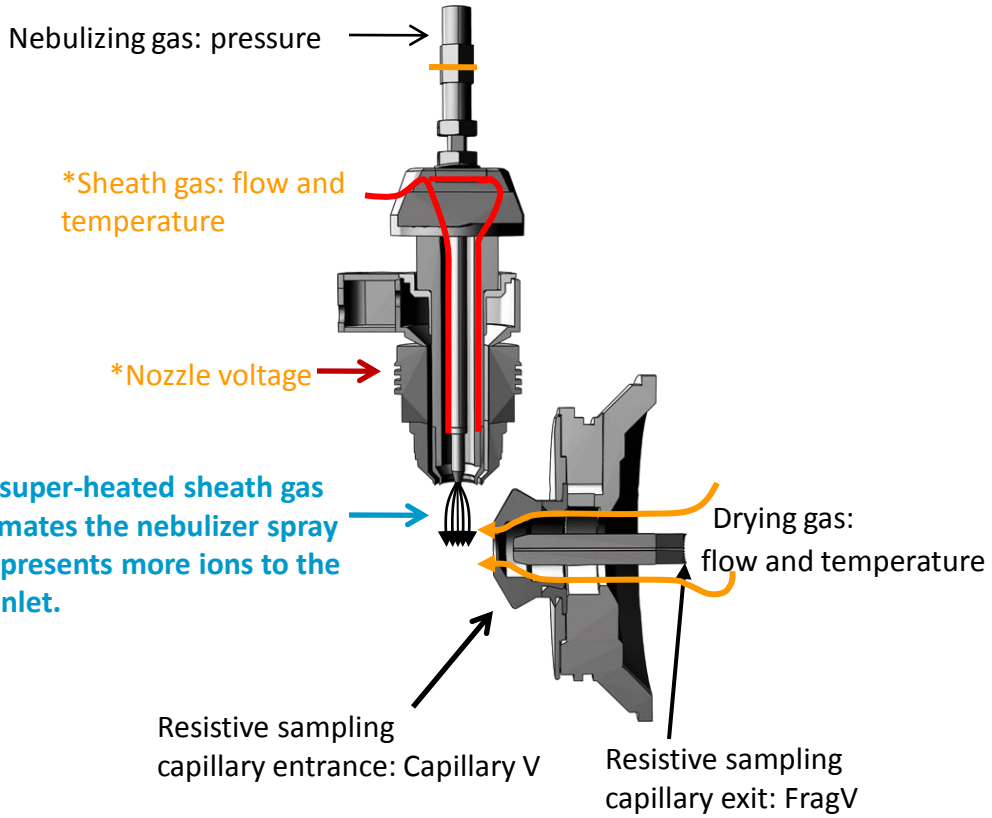
Multimode Spray Chamber Settings



Mode	Nebulizer Pressure (psi)	Drying Gas Flow (l/min)	Drying Gas Temp (°C)
APCI	**30-60**	**5 - 8**	**300-350**
ESI	**30-60**	**8-13**	**325**

APCI/ESI Vap Temp (°C)	Corona Current APCI (nA)	Capillary Voltage (V)
150(ESI) 250(APCI)	**4000** (pos) **& 20,000** (neg)	**3500**

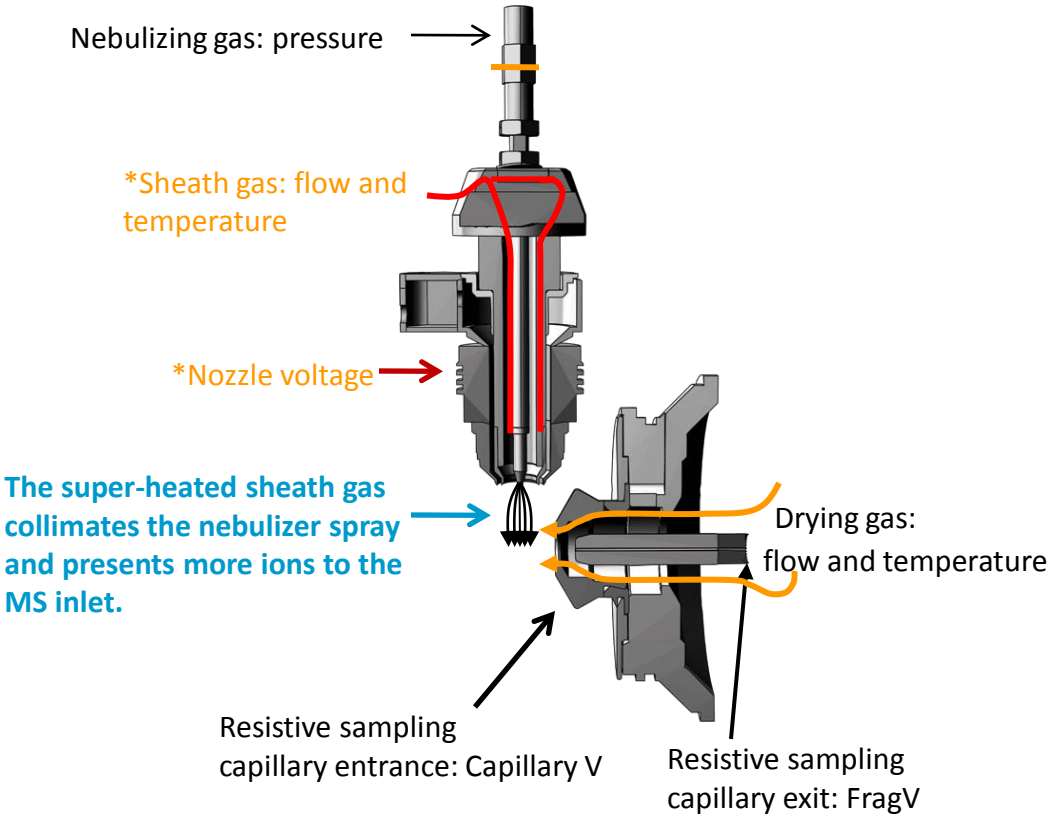
Agilent Jetstream Technology (AJT) source
Non-ion funnel small molecule



Parameter Starting Conditions	Agilent Jetstream Pos/Neg
Nebulizer pressure	35-45 psi
Drying gas flow	10-12 LPM
Drying gas temp	275°C/325°C
Sheath gas flow	10-12 LPM
Capillary voltage	3500V/3000V
Nozzle voltage (AJT)	0V/1500V Pos - low/Neg - higher
Fragmentor voltage (cpd-dependent)	100-175V QTOF/TOF

*New parameters unique to AJT source

Agilent Jetstream Technology (AJT) source
Non-ion funnel intact protein



Parameter Starting Conditions	Agilent Jetstream Pos/Neg
Nebulizer pressure	55 psi
Drying gas flow	10 LPM
Drying gas temp	350°C
Sheath gas flow	10-12 LPM
Capillary voltage	4500-5500V
Nozzle voltage (AJT)	2000V
Fragmentor voltage (cpd-dependent)	225-400V QTOF, TOF

*New parameters unique to AJT source

Note that “quad amu” will need to be increased 100-250amu with old quad drive in quad tuning parameters for QTOF