API Optimization, Source Design, and Other Considerations of LC/MS

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Instrument Design Considerations: The Atmosphere-Vacuum Interface and Pumping System

The hows and whys of the vacuum?

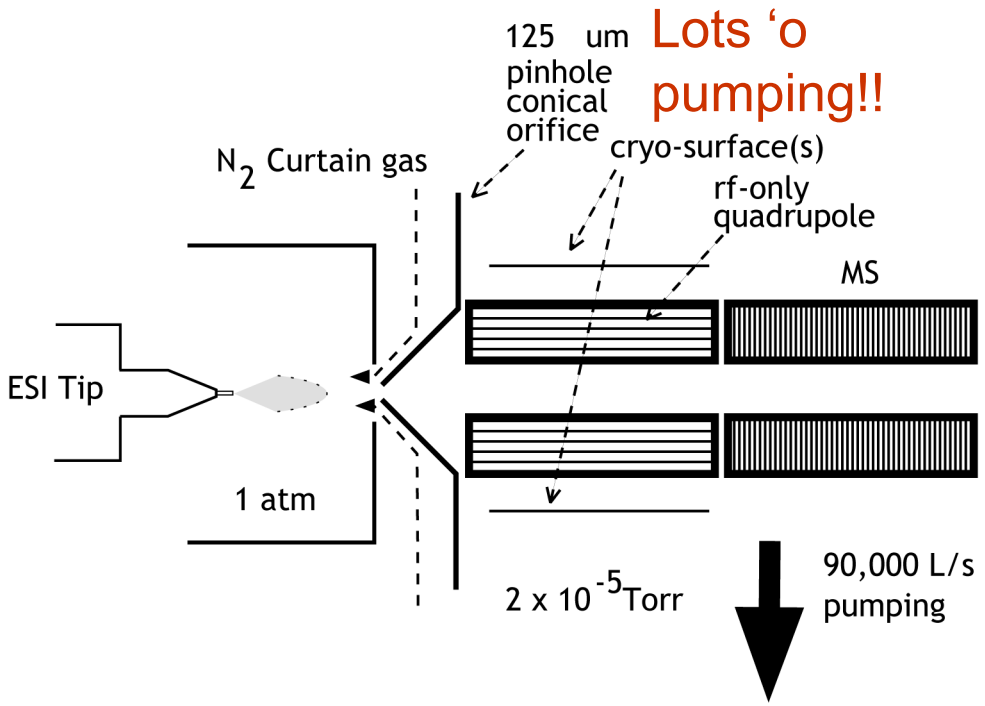
- We've got to get from 760 torr to 10^{-6} Torr
- We need vacuum to focus and transport ions efficiently without scattering losses
- Profound statement: You can never have too much pumping
- Two approaches to vacuum pumping:
  - Single stage pumping
  - Differential (multi-stage) pumping
- A number of different approaches to atm-vac interface design are currently in production
  - Heated capillary, on-axis sprayer (Finnigan API-1, API-2 sources)
  - 90° off axis sprayer (AQA, Z spray, Agilent, TSQ Quantum)
- Different interface designs have different analytical and operational characteristics
  - This presentation will focus on the heated capillary interface, which is used in the majority of Finnigan LC/MS interfaces

Single stage pumping

Basis of PE-Sciex TAGA and API-III Design

Plate orifice interface characteristics

- Interface cannot tolerate high liquid flows along the spray axis
- a "gentle" source



Advantages:

- Pumping!!
- Simple design
- High transport efficiency

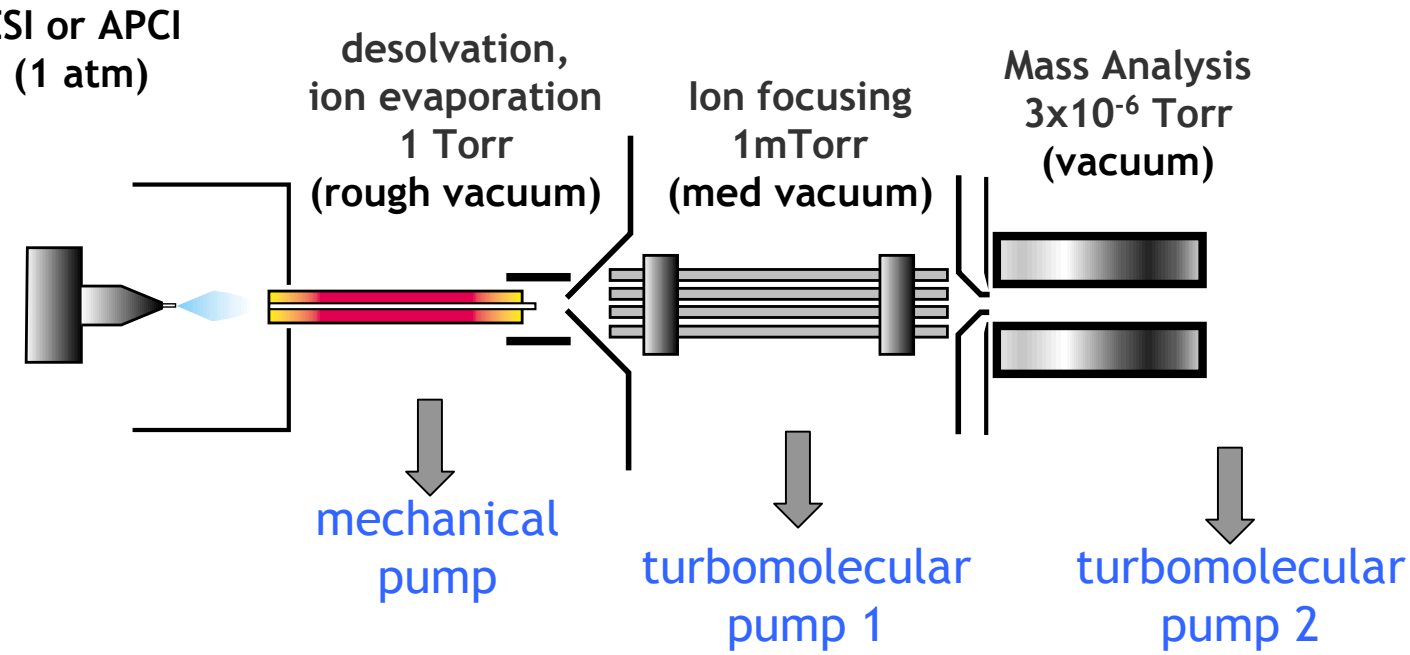
Disadvantages

- Must recycle (thaw) cryo surfaces daily
- Small pinhole
- Noisy pump operation
- Pump is expensive if it breaks

Schematic of a differentially-pumped, heated-capillary API/MS system

Heated capillary interface characteristics

- Can tolerate the widest range of liquid flows
- Excellent for biomolecules
- Thermal degradation is possible with certain compounds but can be minimized



Advantages:

- Larger inlet orifices possible
- 24/7 system
- Turbos are relatively reliable (technology improving and becoming lower cost)

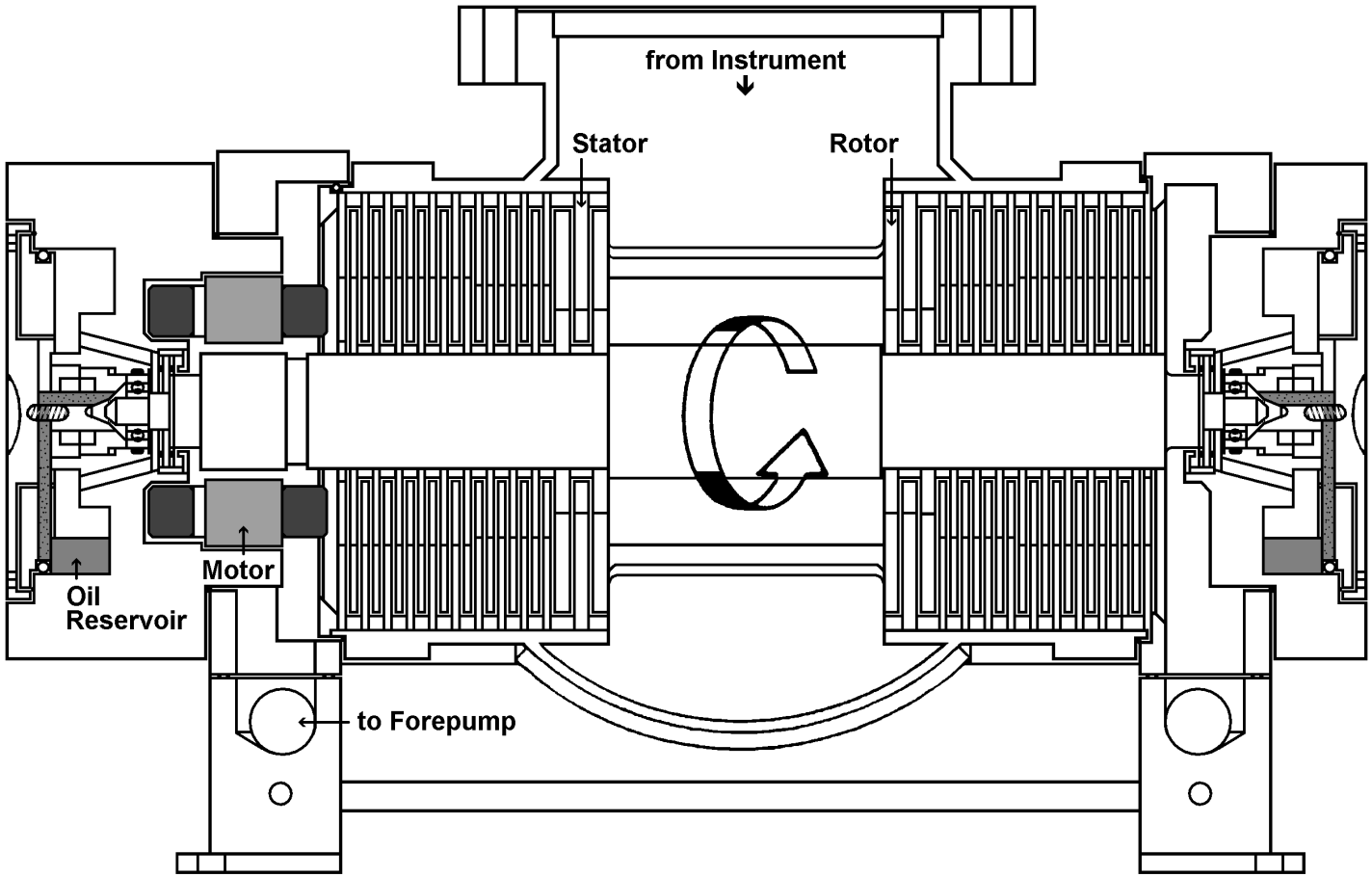
Disadvantages

- Must manage ion transport losses in various stages
- $ increase with # of stages

Cross-section of a turbomolecular pump

Turbo pump characteristics:

- Sounds like a jet engine when spinning up or down
- Operation at high speed 30-60 krmp
- Newer designs have multiple inlet stages and are very low maintenence



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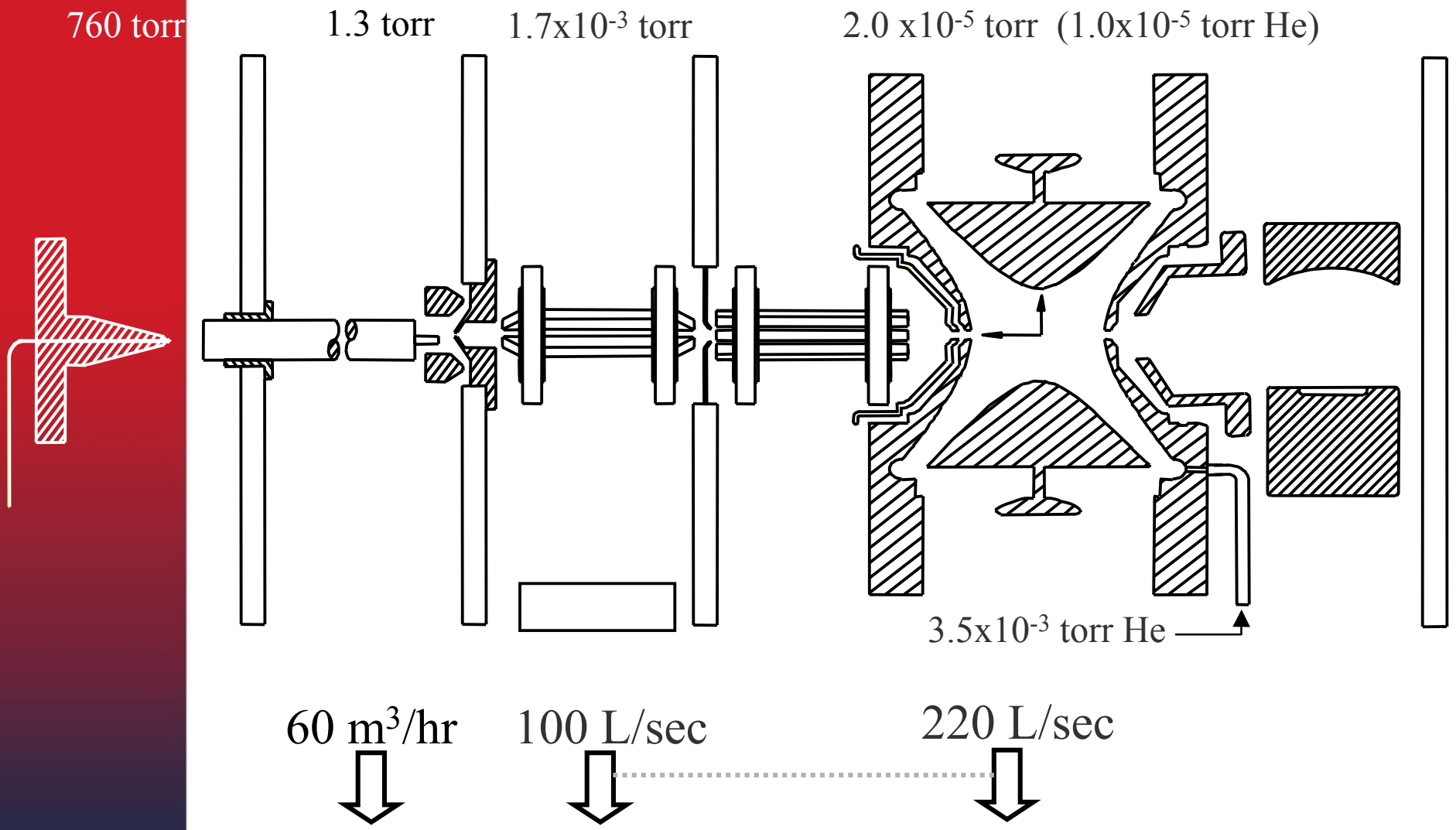
Example: Typical Operating Pressures in an LCQ Deca



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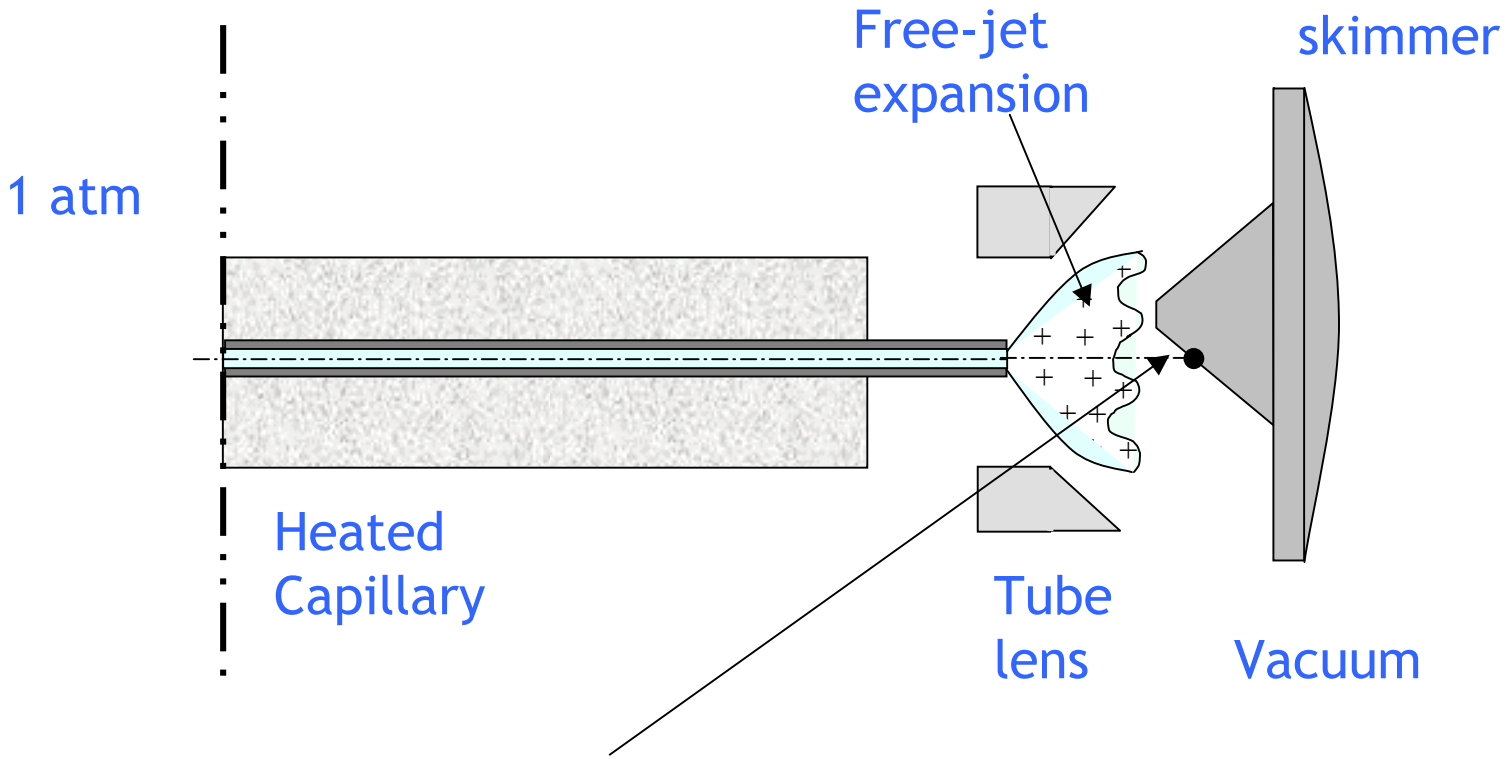
Most API sources operate off-axis with respect to ion sampling to minimize spray-related noise

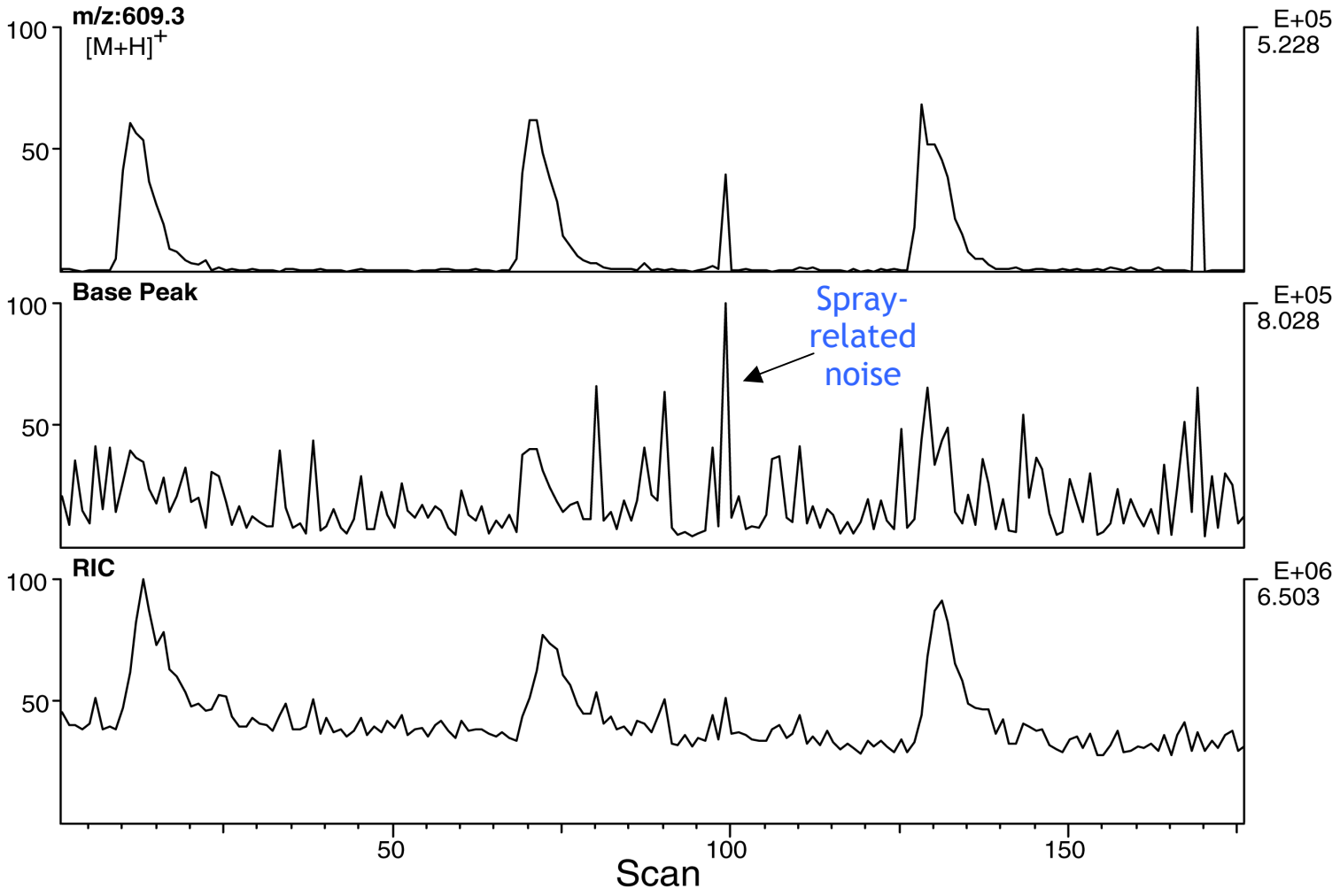
The heated capillary is off-axis with respect to the skimmer in API-1, API-2, and all LCQ source designs



Particles and droplets move to the center of the flow in the jet expansion, impinge on skimmer and are not transmitted, reducing noise dramatically

Design Considerations: on-axis vs. off-axis ion sampling

API-1 TSQ, **capillary-skimmer on-axis**, Reserpine loop inj., 50 uL/min MeOH/H_2O



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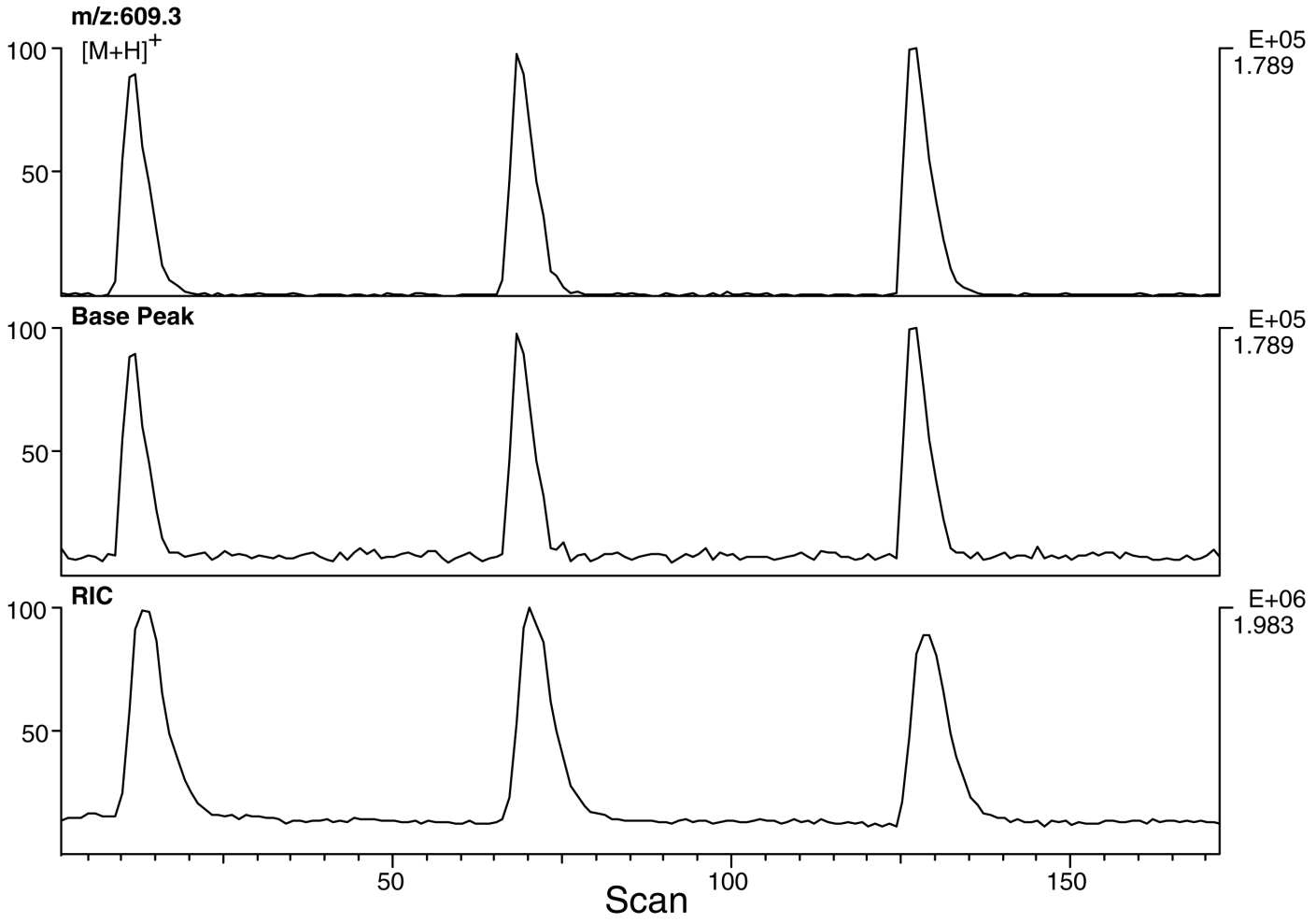
Design Considerations: on vs. off-axis ion sampling

API-1 TSQ, **capillary-skimmer off-axis**, Reserpine loop inj., 50 uL/min MeOH/H_2O



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API Interface Optimization

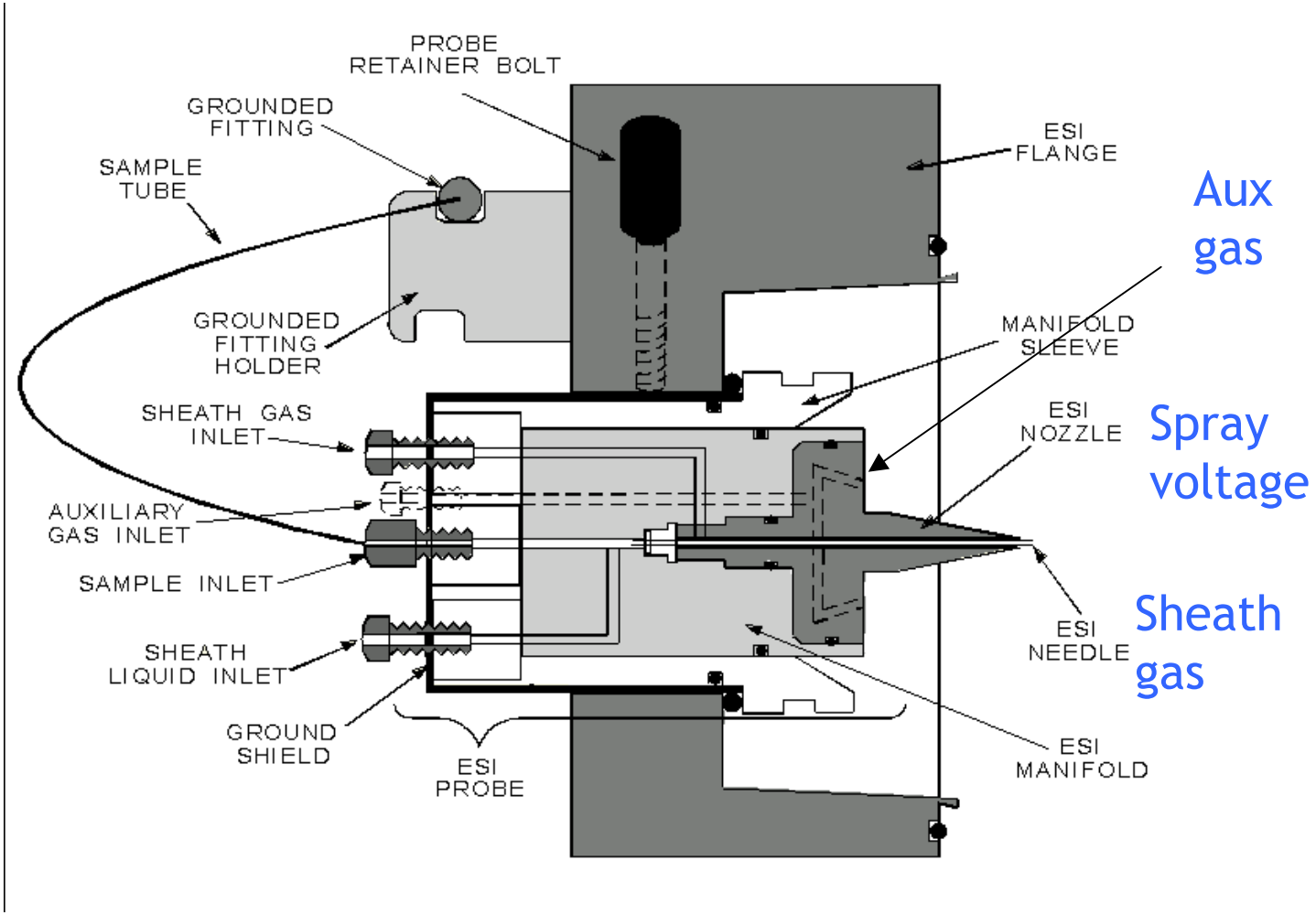
Optimizing Parameters for API/LC/MS

- Interface Parameters
  - Heat (desolvation)
    - Heated capillary, interface plate, etc.
  - N_2 nebulizer or sheath gas (droplet formation)
  - N_2 auxiliary or bath gas (humidity control)
  - Voltages
    - Spray (HV for droplet formation in ESI)
    - Corona (discharge in APcI)
    - Interface (used to optimize ion transport)
- HPLC Parameters
  - Flow rate
  - Buffer (use volatile!)
  - Column diameter
  - Column chemistry

Interface Optimization Rules of Thumb

- Refer to manufacturers "tip sheet" as a starting point
- Some parameters are dependent on the input liquid flow rate
  - Nebulizer gas ("set it and forget it" above 10 uL/min)
  - Auxiliary gas (increase with flow rate, off at low liquid flows)
  - Interface temperature (increase with liquid flow rate)
  - Interface voltages may need to be increased with flow rate, but temperature may accomplish the same task
  - ESI spray voltage (only critical for low flow or nanospray applications)
  - ESI electrode – capillary entrance distance (move closer in low flow operation)
  - Corona voltage (set it and forget it)

Cross-section of ESI source head



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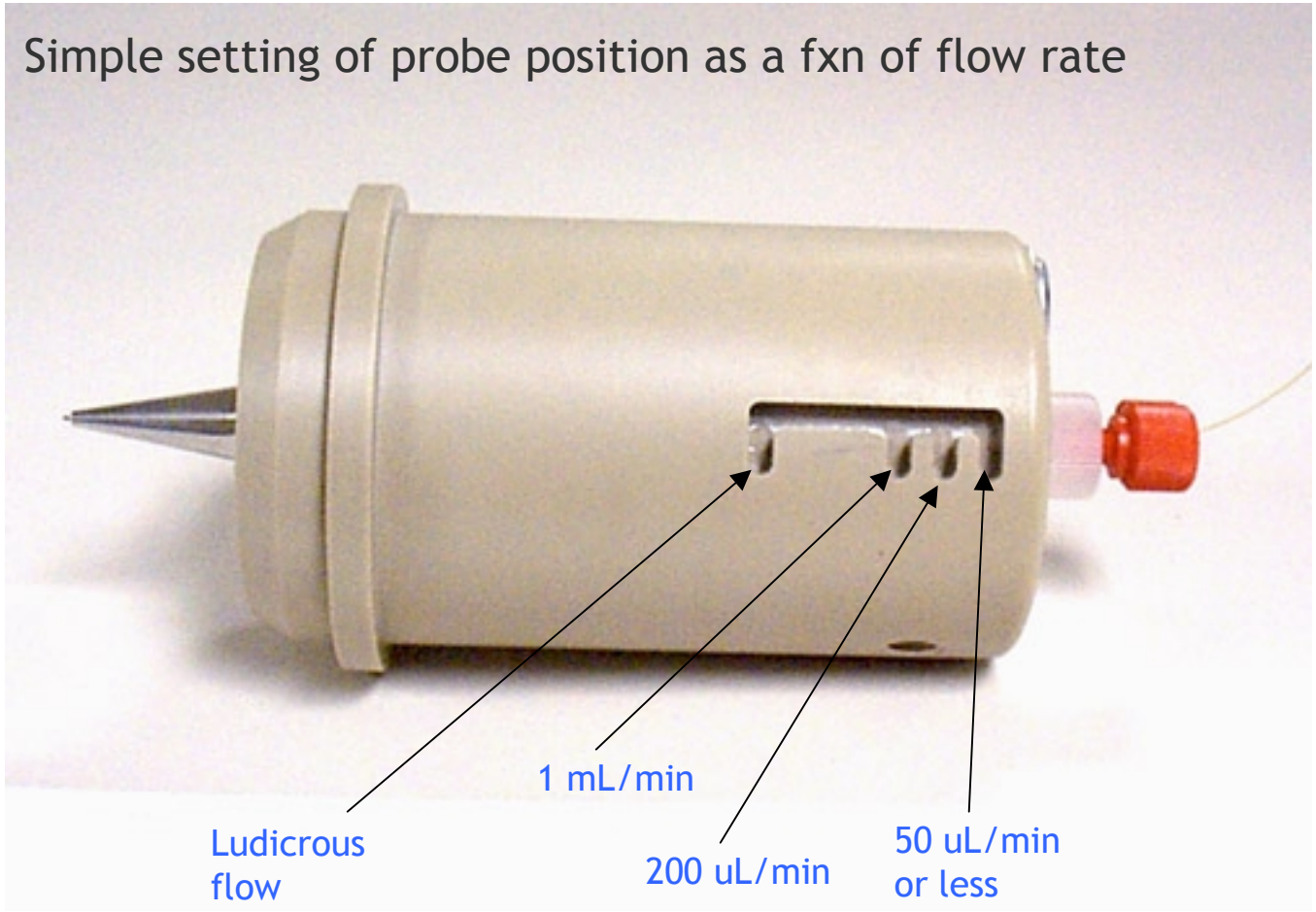
Interface Optimization Rules of Thumb

- Some parameters are dependent on the compound or chemistry!
  - Very thermo-labile or fragile molecules may require:
    - Lower interface temperatures
    - Lower interface voltages
  - Molecules that have affinity for other solvent molecules may require:
    - Higher interface temperatures
    - Higher interface voltages
    - Use of auxiliary gas

Pre-indexed ESI probe on API-2



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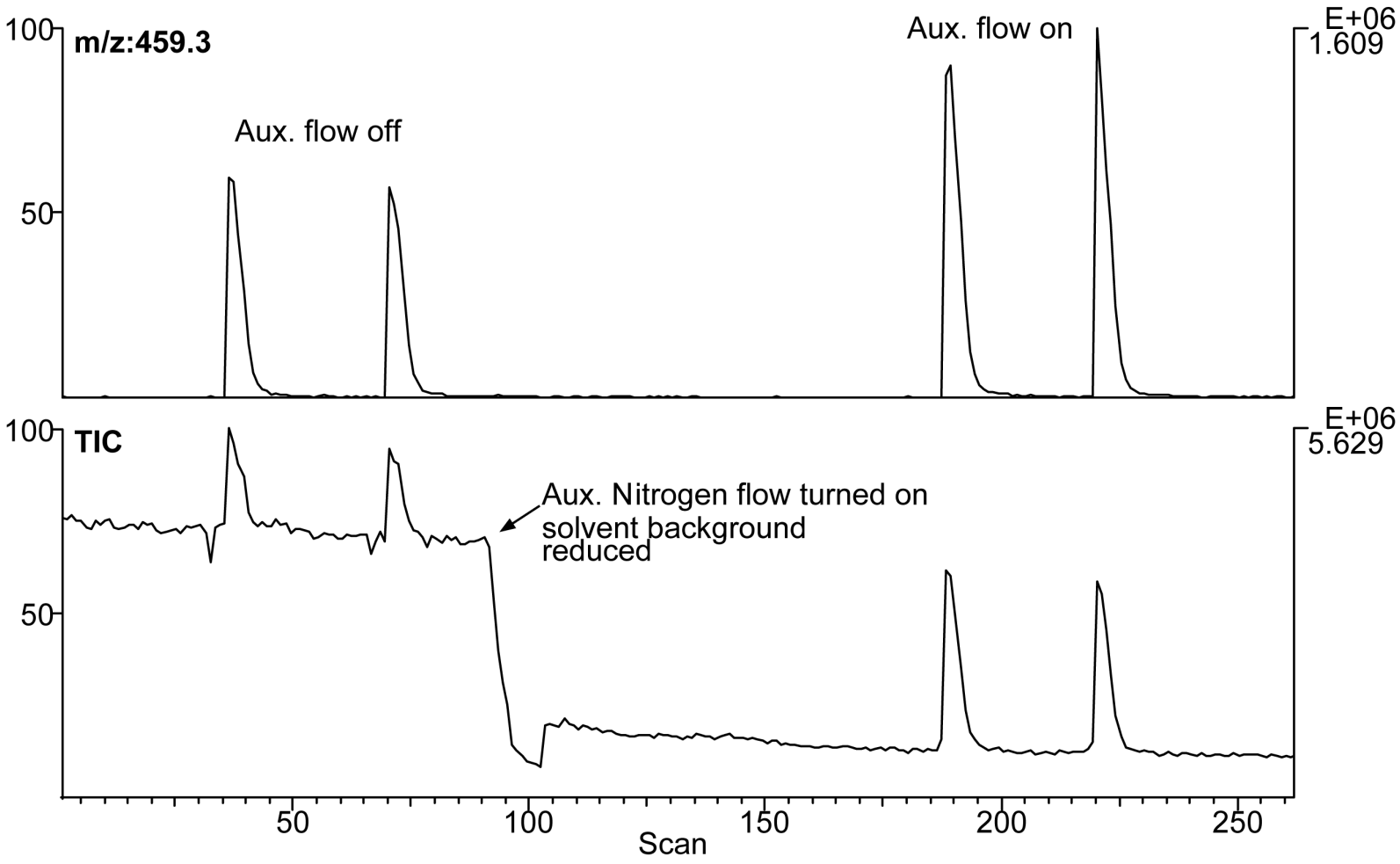
ESI: Effect of Auxiliary N2 Flow on Solvent Background

API-1 TSQ, Astemizole loop injections 200 uL/min
80/20 MeOH/H_2O 10mM NH_4OAc



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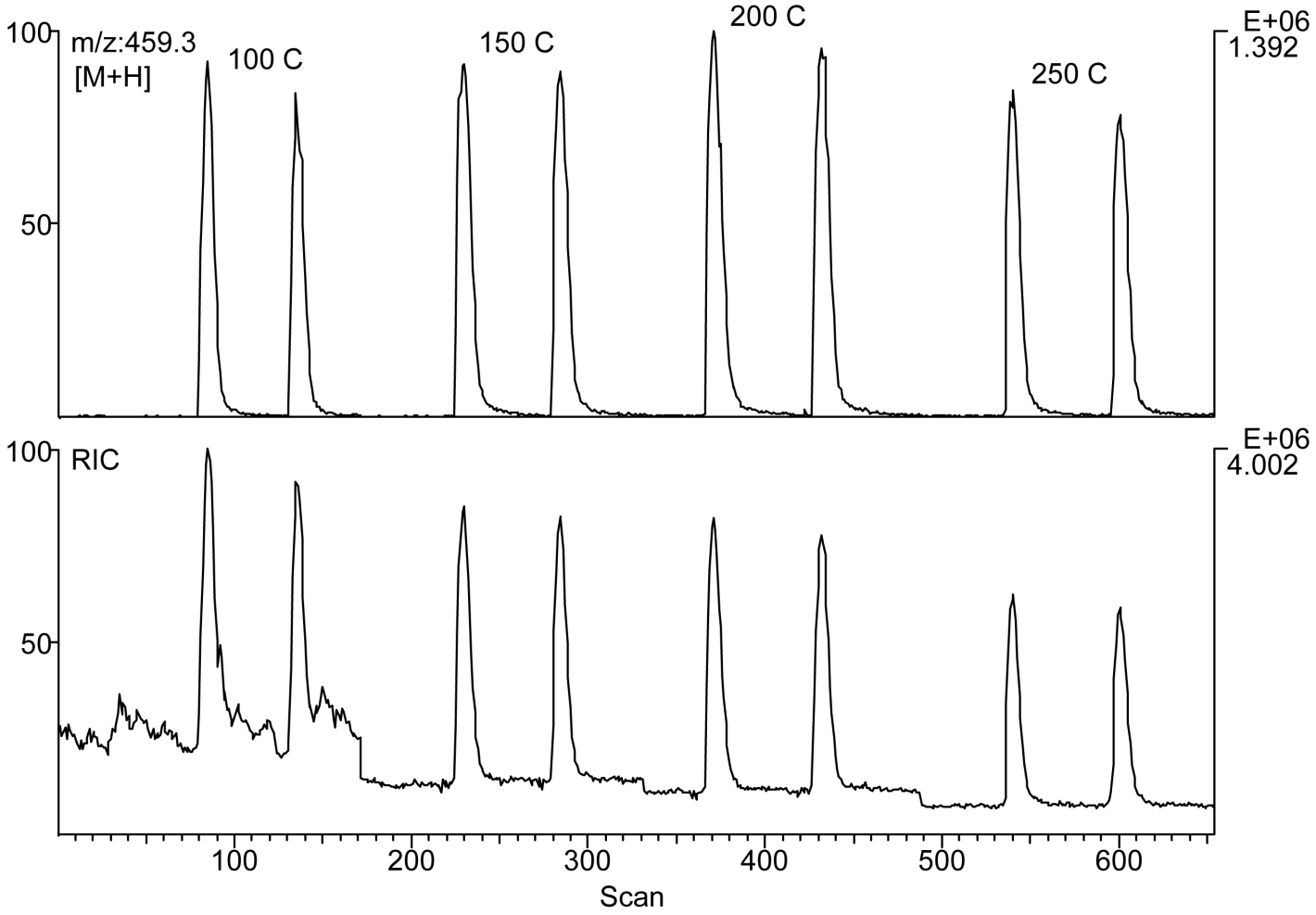
ESI: Effect of Capillary Temperature on S/N at 50 uL/min

API-1 TSQ, Astemizole loop injections 50 uL/min
80/20 MeOH/H_2O 10mM NH_4OAc



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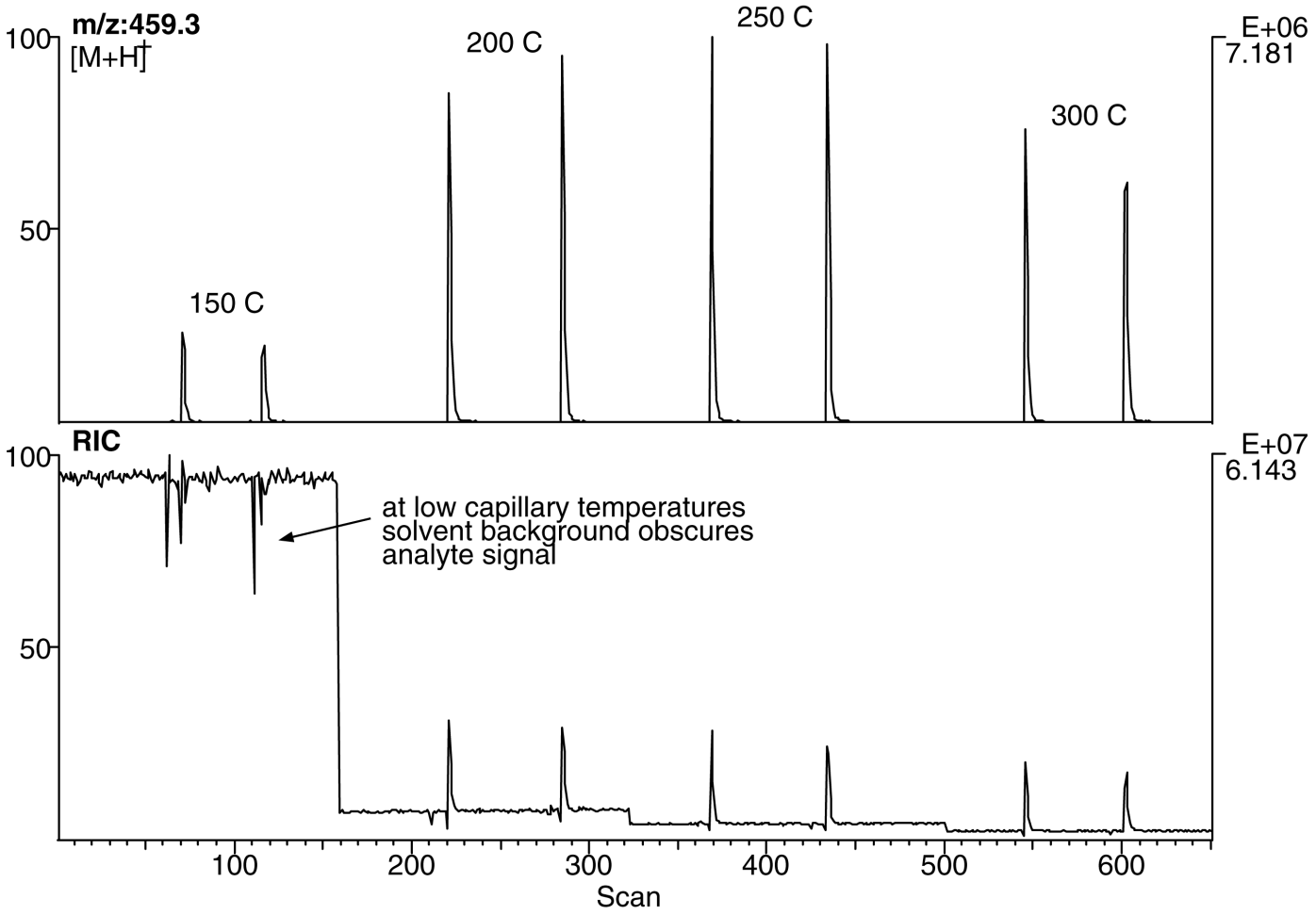
ESI: Effect of Capillary Temperature on S/N at 1 mL/min

API-1 TSQ, Astemizole loop injections 1 mL/min
80/20 MeOH/H_2O 10mM NH_4OAc



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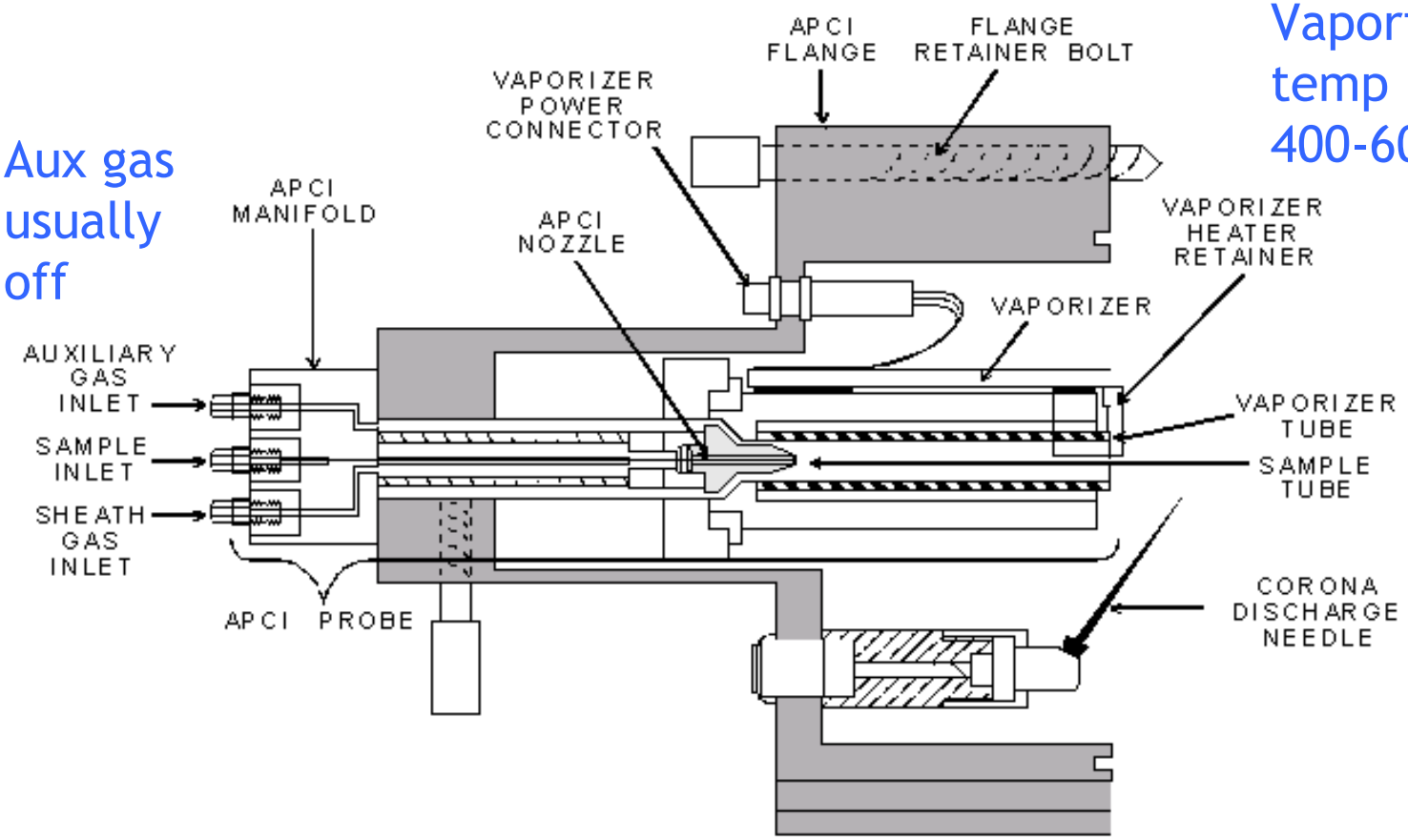
Cross-Section of APcI source head

Aux gas usually off

Vaporizer temp 400-600C



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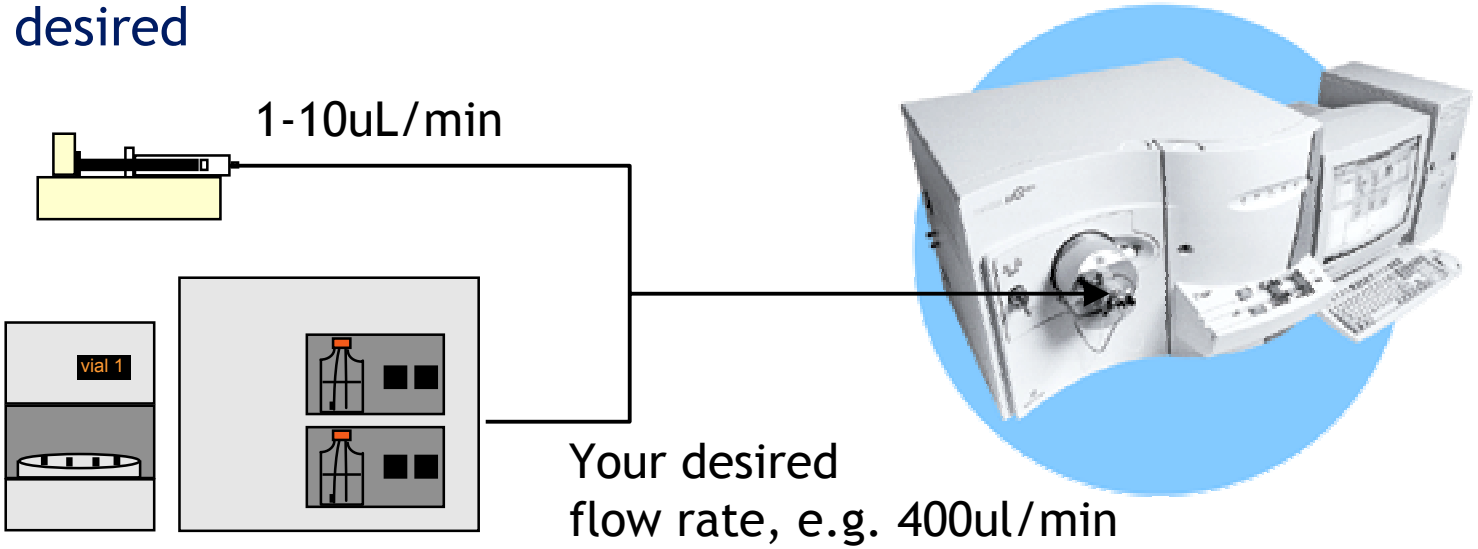
Compound dependent optimization strategies

- For example, optimizing on a target compound for quantitation purposes
  - Tee sample into HPLC stream using infusion pump
  - Monitor analyte signal and optimize each MS parameter as desired



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Optimization strategies, continued

- Teeing in:
  - Advantages are extensive time for tuning and "tweaking"
  - Disadvantage is possibility of interface contamination with your target compound
- Other options:
  - DAC scan or parameter ramp. Finnigan TSQ allows for scanning parameters during loop injection. This is often faster and leads to less interface contamination.

Example Optimum ESI Parameter Matrix for API-1 Source

Liquid Flow Rate	Capillary Temp.	Spray Voltage	ESI-Cap Distance	Sheath Gas	Aux Gas
<10 ul/min	150-200 C		< 0.5 -1 cm	>40	off
50 ul/min	200 C	4.5 kV	1 cm	70 psig	off- 10 units
200 ul/min	225-250 C	4.5 kV	1.5 cm	70 psig	20 units
1000 ul/min	250-300 C	4.5 kV	1.5 cm	70 psig	30+ units

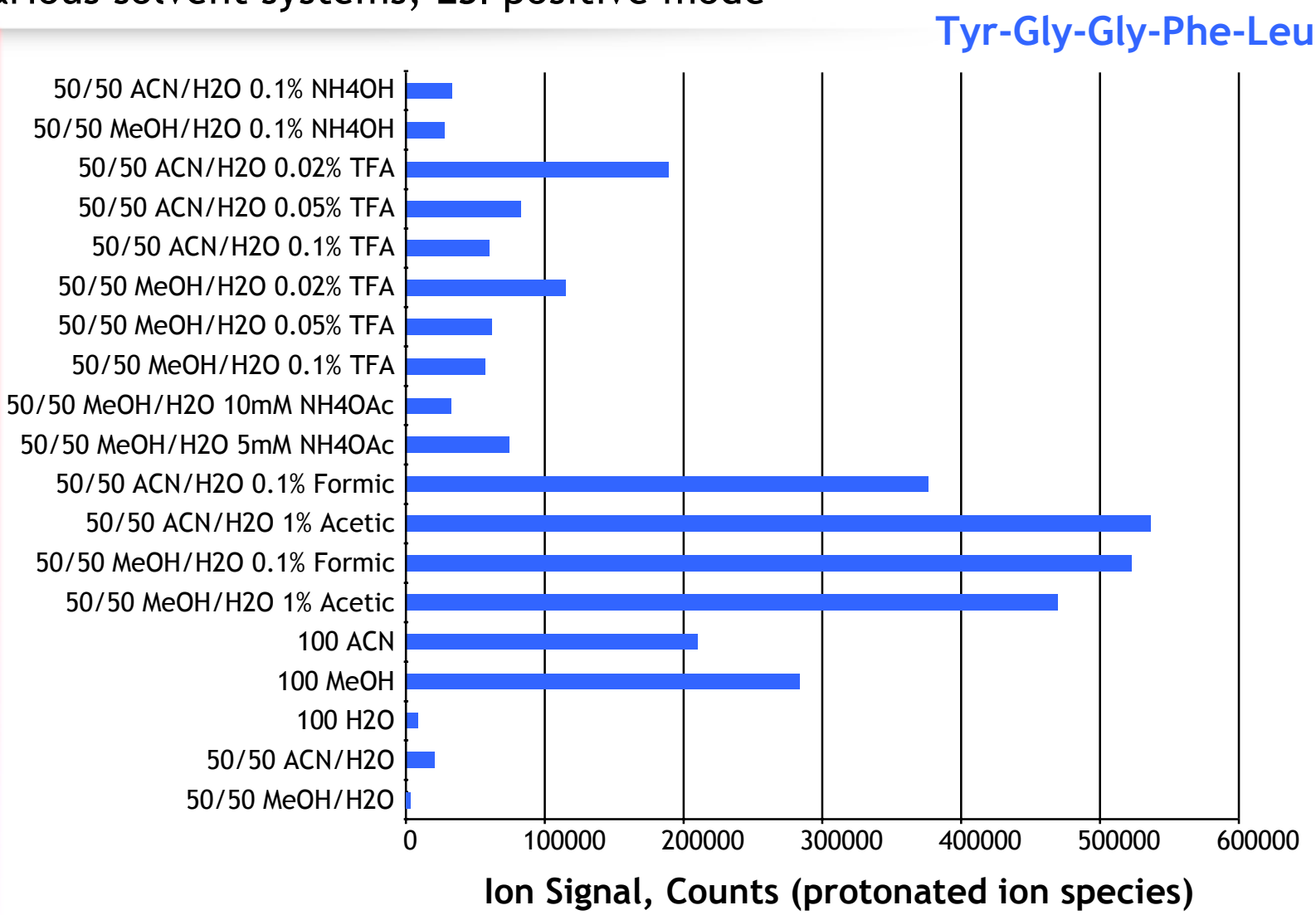
Optimizing HPLC Conditions for API Operation

HPLC buffer optimization notes

- Remember: ESI is a process that takes pre-formed ions in solution and "pops" them into the gas phase
  - Try to pick a buffer that will promote the ionization of your compound
  - The above does not always allow for optimum chromatography, there may be tradeoffs
  - Stick with volatile buffers if possible
  - Stick with low ionic strength buffers if possible
  - Some modifiers suppress signal (e.g., TFA)
  - New column technology is reducing the requirements of modifier use
  - Modifiers in APCI have less of an effect since ions are formed from vaporized sample via chemical ionization

Example of solution chemistry effects: Leu-enkephalin Signal with various solvent systems, ESI positive mode

Solvent System



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Picking a column diameter

- Not sample limited (impurity profiling, process, QC)
  - 2 – 4.6 mm i.d.
- Somewhat sample limited (peptide digests, *in vivo* metabolite isolates, pharmacokinetics studies)
  - 1-2 mm i.d.
- Sample limited (peptides, extensive multi-experiment studies on a single sample)
  - 0.2 – 1 mm i.d.
- Severely sample limited (in-gel protein digests, real-time *in-vivo* monitoring)
  - 0.05 – 0.1 mm i.d.

Column Arsenal for LC/MS

- Qualitative Small Molecule Analysis
  - You may want to choose a variety of chemistries
    - Stable column for low pH (TFA) work (e.g., Zorbax SB-C18)
    - Polar compounds in highly aqueous mobile phases (e.g., YMC-AQ)
    - Negative ion work stable column at pH 6-7 for (e.g., Luna C18)
    - Base-deactivated for low or no TFA work (e.g., MAGIC C18)
- High-throughput and/or quantitation
  - Choose columns of short length (5 cm or less) and small particle diameter (3-5 um) (e.g., MAGIC bullets)
  - Use in-line filters!
  - As in qualitative analysis, may be useful to have a variety of column chemistries on hand
- Peptide
  - Use a good base deactivated 300A C18 column, leave out the TFA if you need ultimate sensitivity (e.g., MAGIC C18)
  - Capillary columns for proteomics applications (often home-packed)
- Protein
  - large pore polymeric, 5 cm length (e.g., PLRP-4000A)

The Issue of Flow Splitting

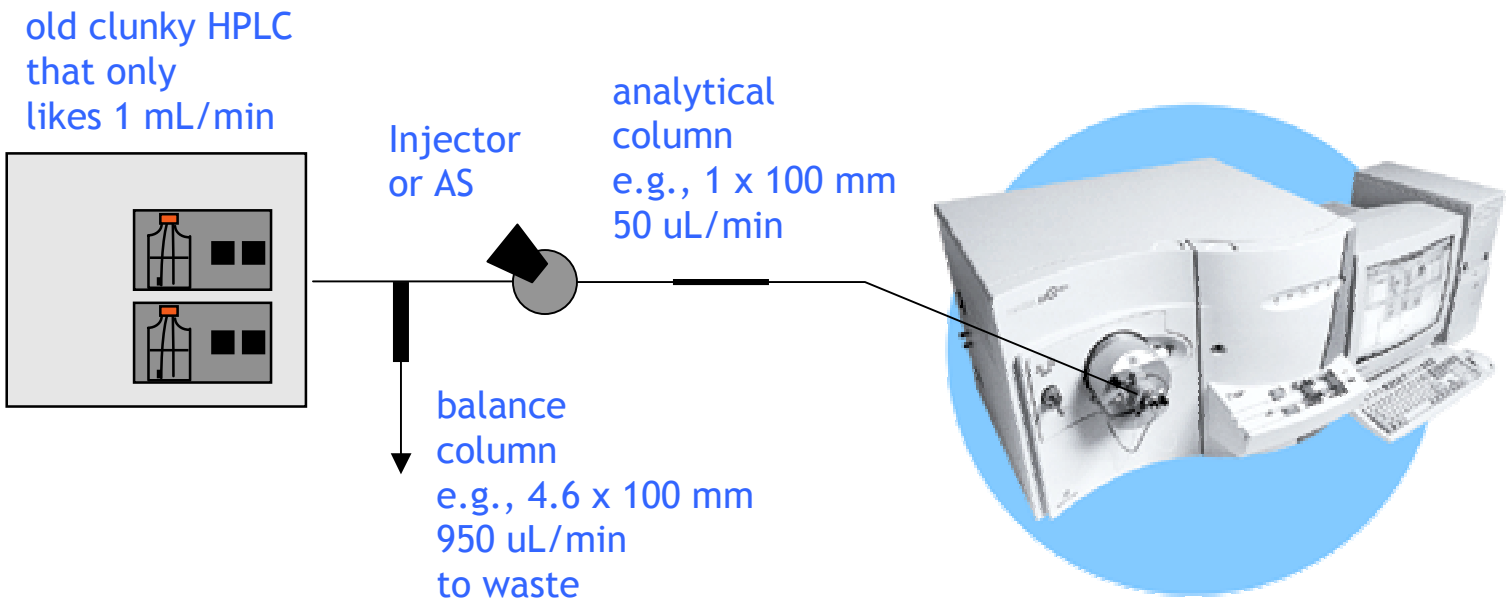
Pre-Column
Post-Column

For ESI, do you need to split the LC flow to obtain optimum performance?

- But wait…there are 2 kinds of splits
  - Pre-column, pre-injector (to use a smaller i.d. column)
  - Post-column (to reduce the total flow into the MS)
- Need for post-column split is dependent on the source design
  - Heated capillary sources offer compatibility of the entire liquid flow range (<1 – 1000 uL/min)
  - Post-column splitting is often recommended for other designs (consult the manufacturer)
- Maybe you want to split!
  - Post-column: data-dependent fraction collection or purification
  - Post-column: since ESI is roughly concentration-dependent you won't loose a significant amount by splitting
  - Pre-column: you want to do capillary or nano LC

How do you split if you need to? Example: Pre-column splitting for micro-column operation

Columns of the same length and packing will give ~the same pressure drop if operated at their optimum flow rates. Therefore a split based on the cross-sectional areas of the columns will be created. E.g., for 4.6:1 mm ~ 20:1 split



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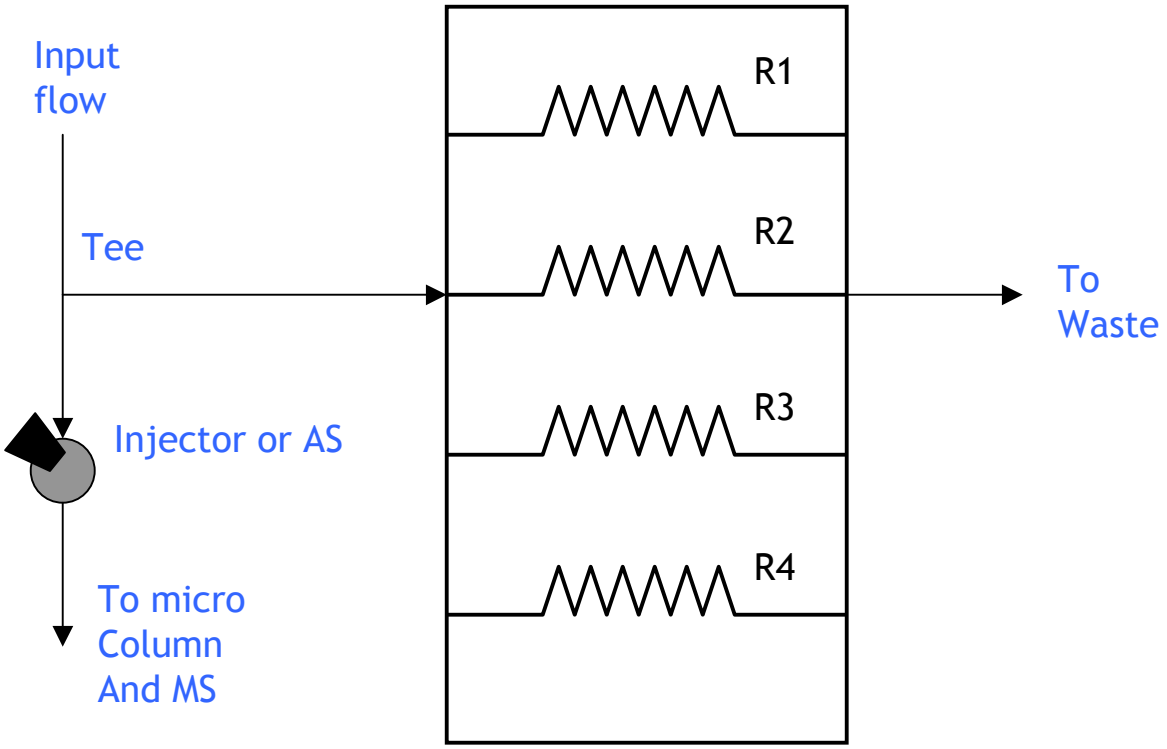
Variable pre-column splitters

Designed to give a particular pressure drop at a given input flow rate. Select a "resistor" to give you the right flow. Example: Michrom Variable Pre-column Splitter Assembly. These are very convenient, particularly for capillary column applications.



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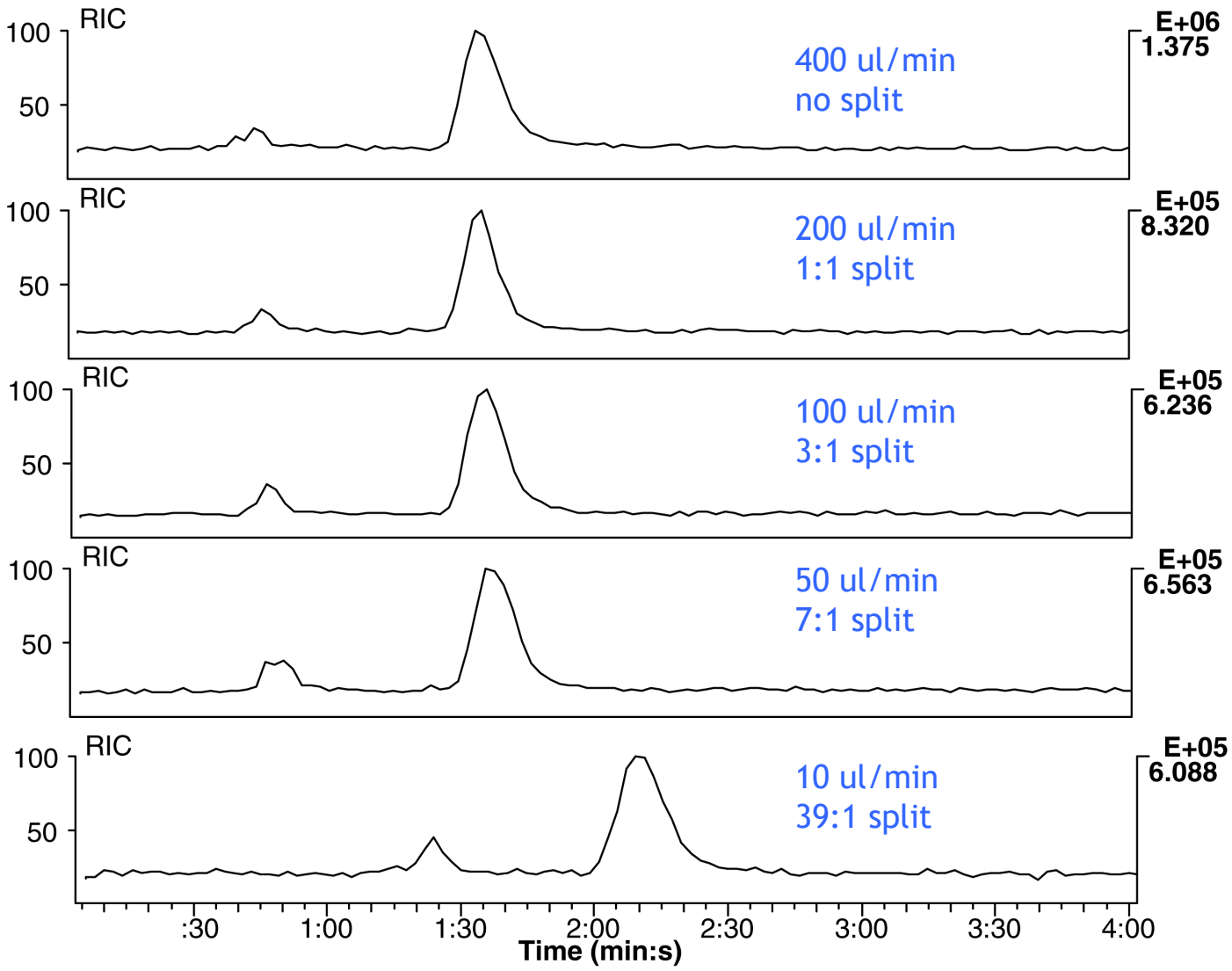
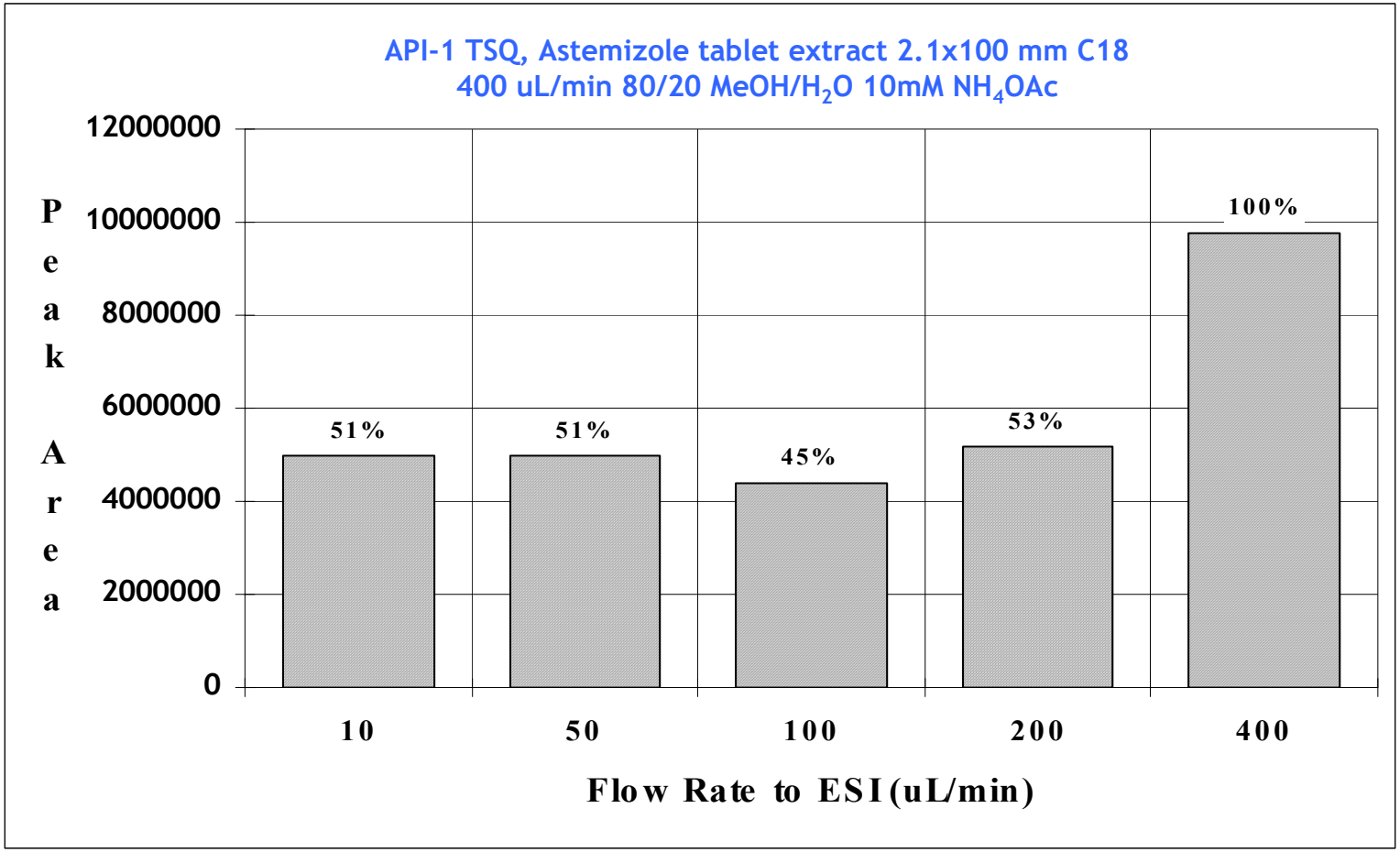
ESI: Effect of Post-Column Flow Splitting on Performance

API-1 TSQ, Astemizole tablet extract 2.1x100 mm C18, 400 uL/min 80/20 MeOH/H_2O 10mM NH_4OAc



Effect of post-column splitting on peak areas

API-1 source on TSQ shows fairly constant signal, regardless of flow to ESI/MS, but response is higher without splitting?



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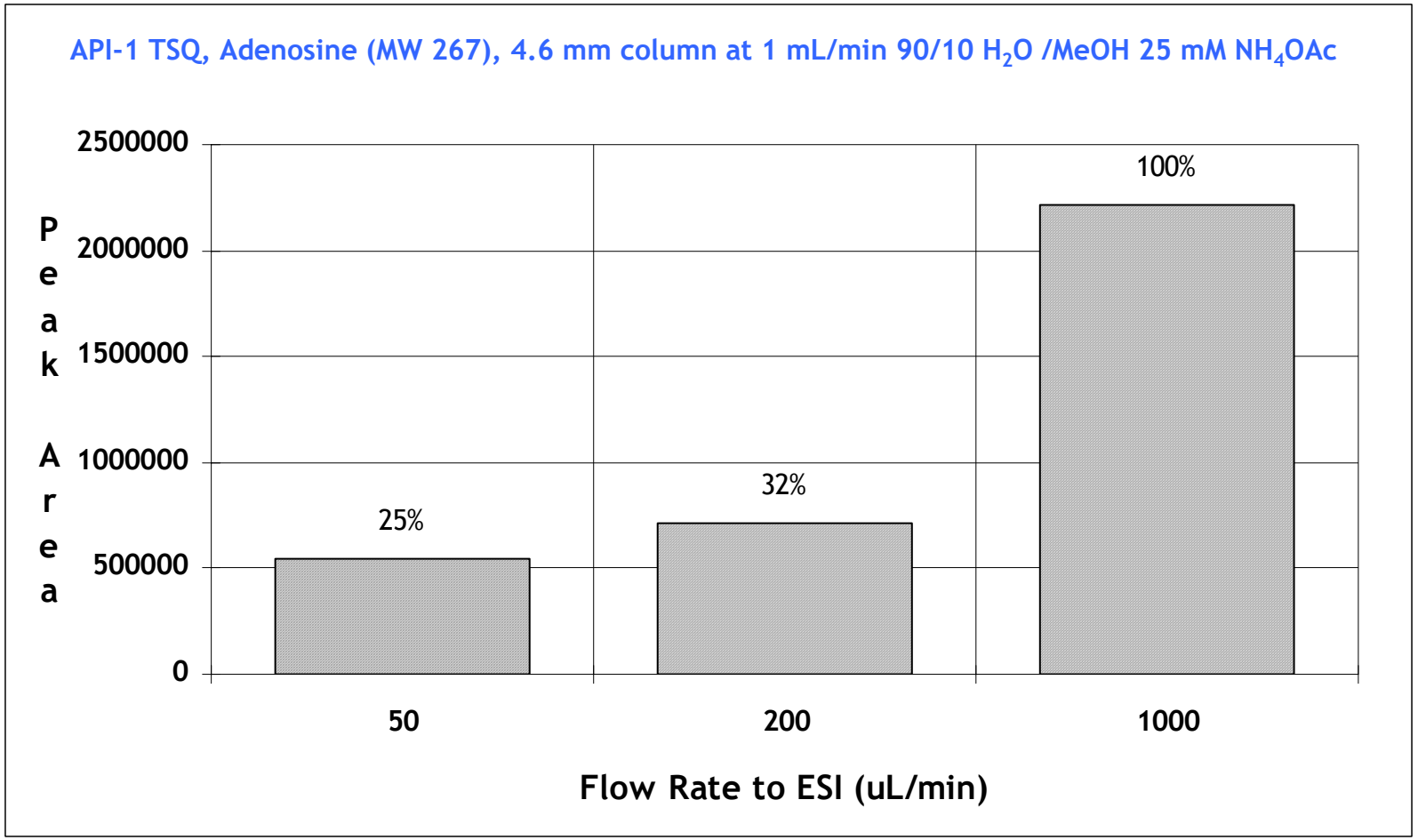

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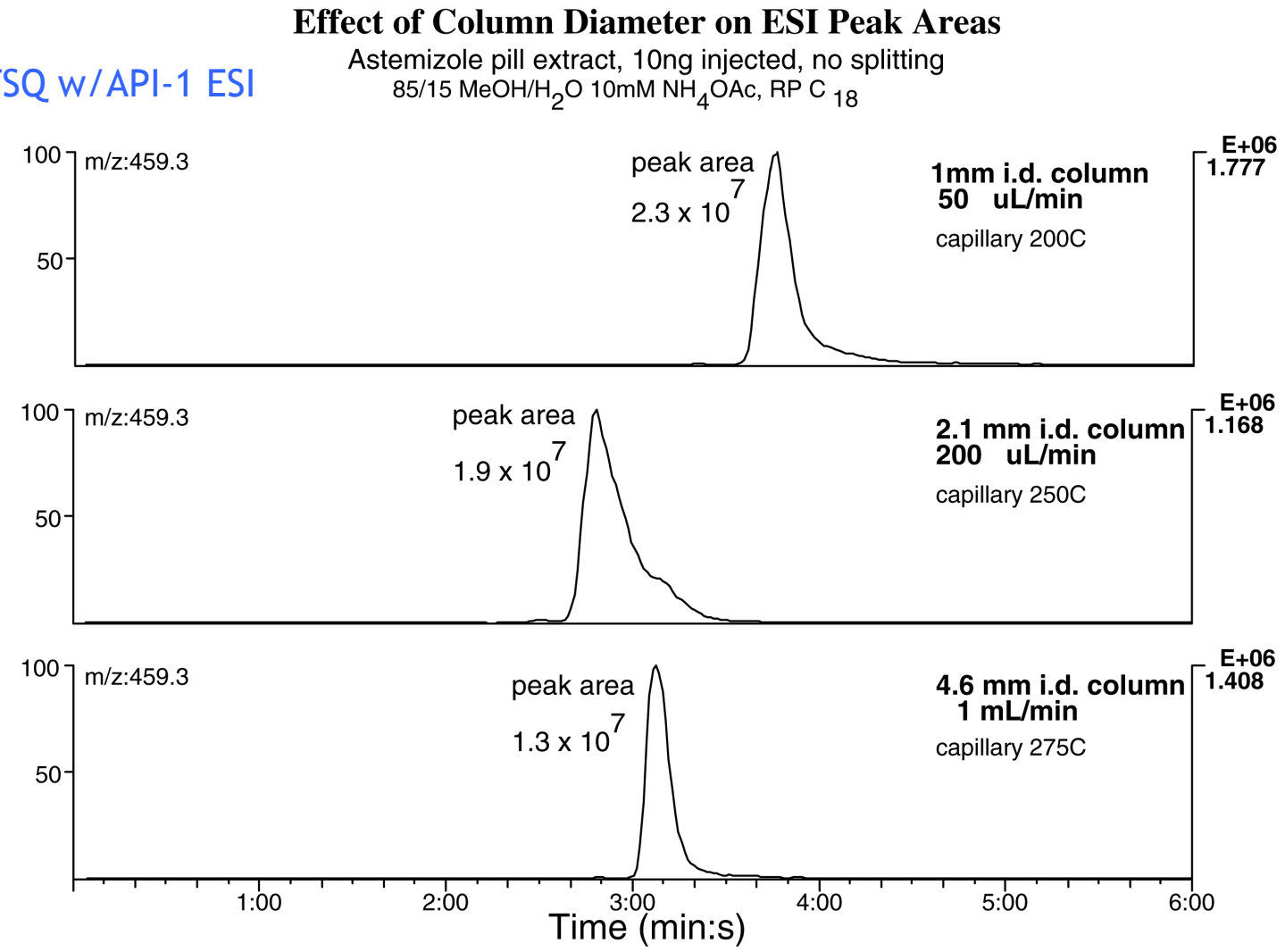
Effect of Column Diameter on Peak Areas

TSQ w/API-1 ESI



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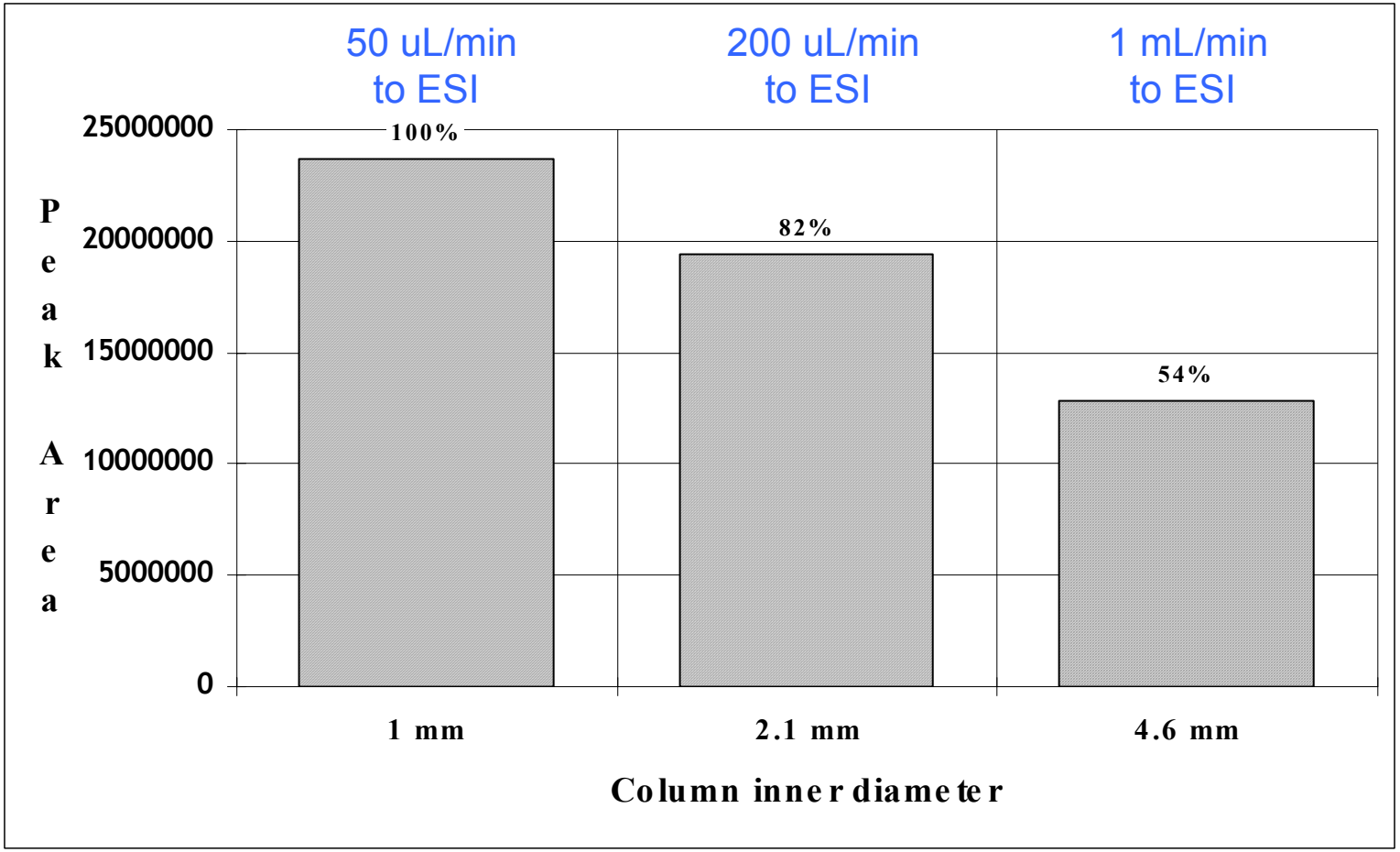
Effect of Column Diameter on Peak Areas

Smaller column diameters result in higher concentrations at the detector and increased peak areas, but not as much as expected?



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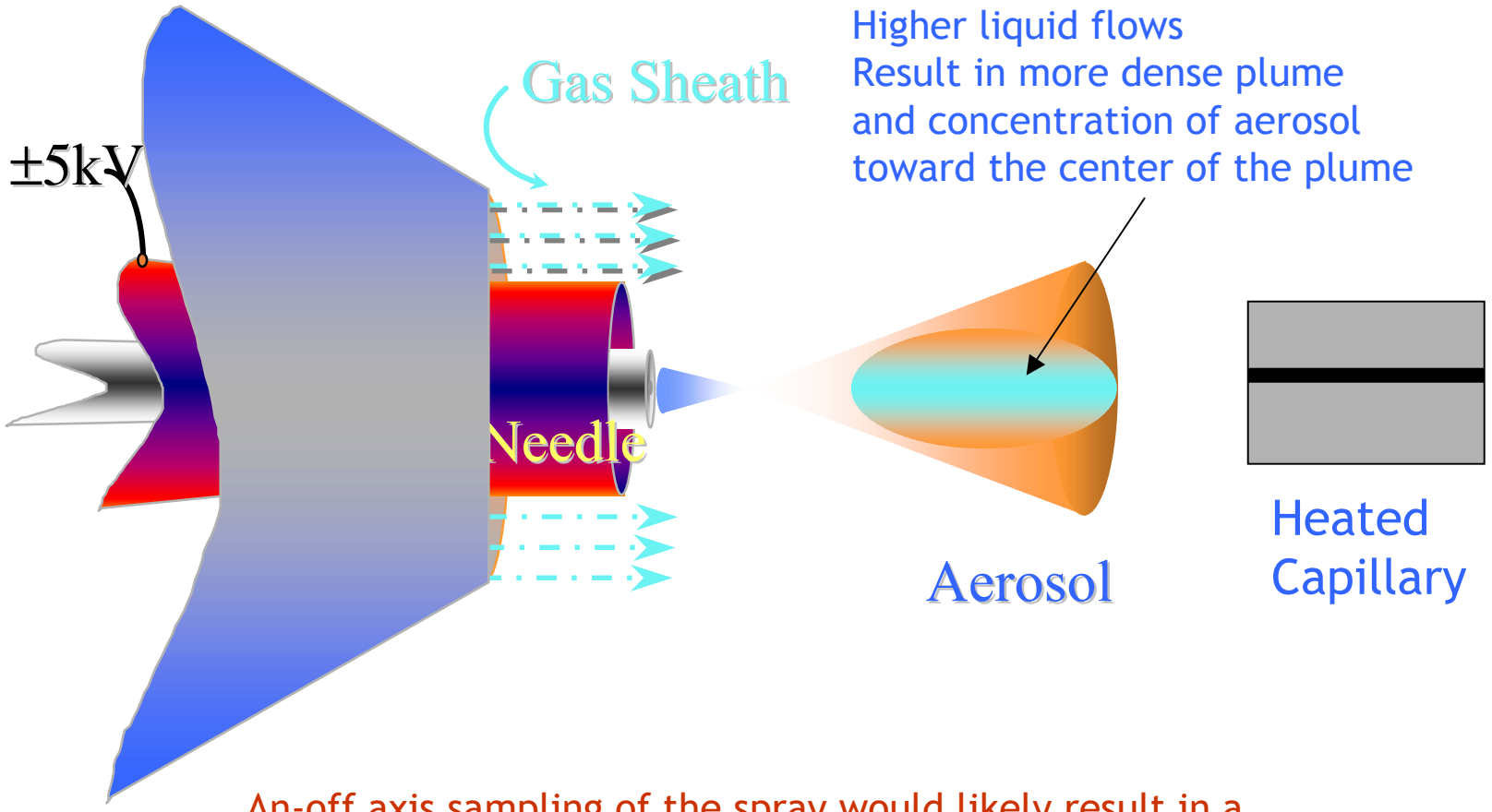
Possible explanation for "not-so-concentration-dependent behavior" of Finnigan Source Design



An-off axis sampling of the spray would likely result in a purely concentration-dependent device

Splitting issues

- Pre-column, pre-injector splitting can be used to couple conventional HPLCs to small bore columns.
- In an optimized heated-capillary API interface, there is no need to flow-split post column unless sample collection is desired.
- Response in ESI is a fxn of analyte concentration.
- Better mass sensitivity will be obtained with smaller column diameters and lower flow rates.
- On-axis heated-capillary interface has interesting response at high flow rates. It appears that aerosol density may result increased sampling efficiency at higher flow rates.