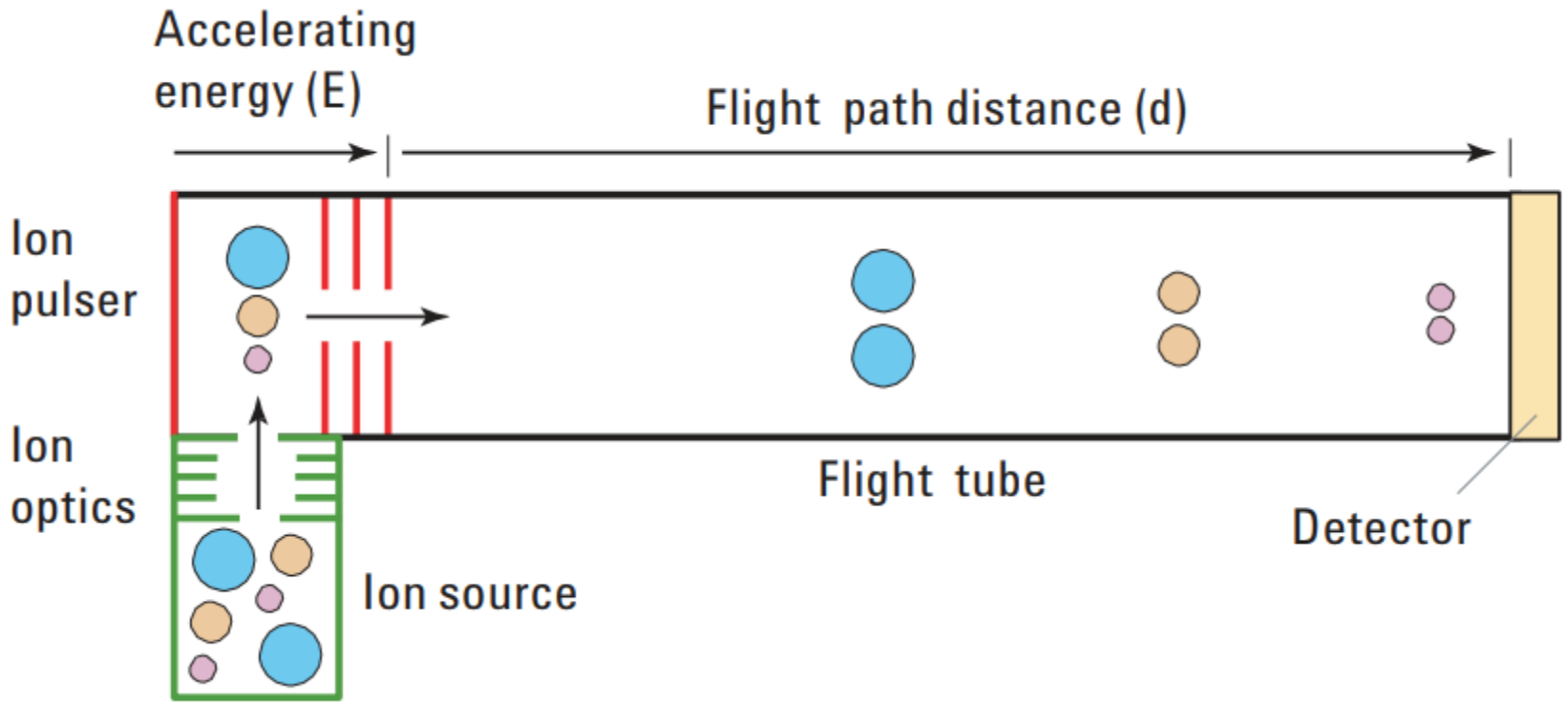
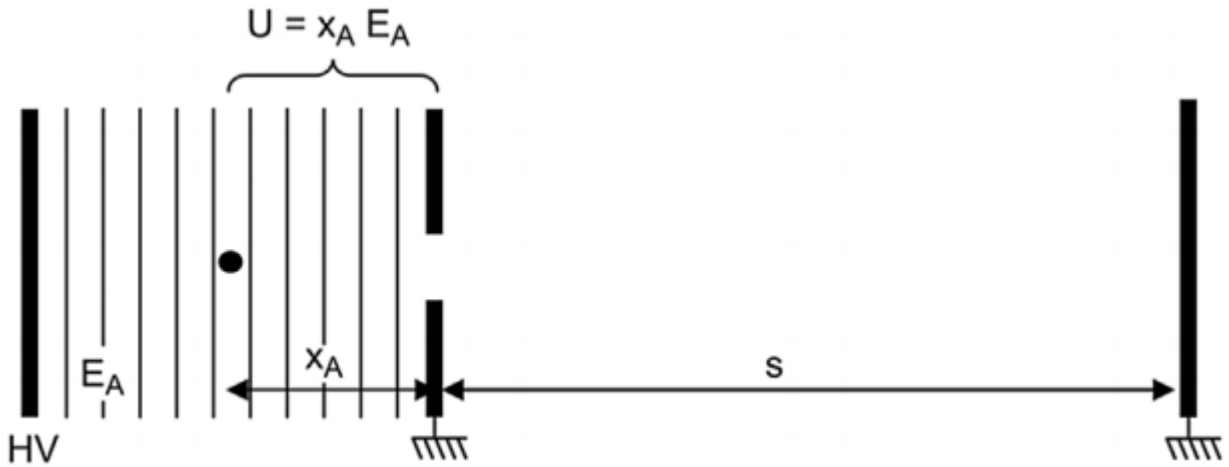
# Time-of-flight mass analyzers



# Advantages of TOF mass analyzers

- high mass resolution
- no limitation is mass range
- easily coupled to various ion sources and mass analyzers
- relatively simple design




flight time of analyte ion

$t_{acc} \ll t_{drift}$

flight time in acceleration region

$$E_{kin} = \frac{1}{2}mv^2 = \mathrm{q} \times \mathrm{U} \qquad acceleration = \frac{qE_A}{m}$$

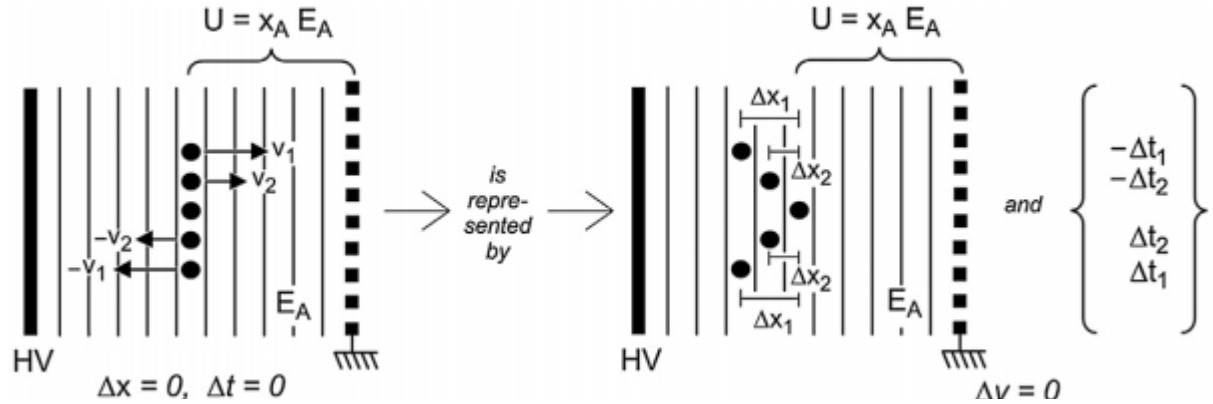
$$t_{acc} = 1.44\sqrt{x_A} \times \sqrt{\frac{m}{E_A}}$$

flight time in field-free region

$$t_{drift} = \frac{s}{v} = s\sqrt{\frac{2qU}{m}}$$

Boesi U. Mass Spectrom Rev 2017; 36:86.

# Error terms in $E_{kin}$: initial distributions of Δv, Δx, Δt → result in **flight time incertitude**




| | | |
|---|---|---|
| $\Delta t$ | $Dt_t = \Delta t$ | Pulsed extraction field $E_A$<br>Short ionizing laser pulses (< 1 ns)<br>Fast response time of ion detector |
| $\Delta x$ | $Dt_x/t = 1/2\ \Delta x_A/x_A$ | Weak extraction field $E_A$<br>Space focus (see below) |
| $\Delta v$ | Pseudo spatial error<br>$Dt_{v\text{-}x}/t = -\ 1/2\ U_i/U$<br>Pseudo temporal error<br>$Dt_{v\text{-}t} = m\Delta v_i/qE_A$ | Vertical effusive molecular beam[a]<br>Supersonic molecular beam[b]<br><br>Strong extraction field $E_A$[c] |

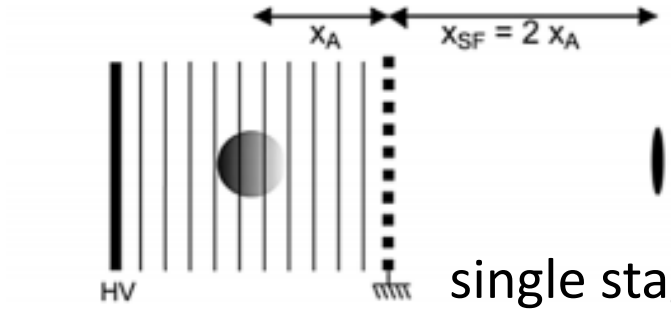
[a] vertical to ion extraction; [b] small initial $\Delta v$; [c] problem with $\Delta x$–compensation

Boesi U. Mass Spectrom Rev 2017; 36:86.

# The ion detector is optimally positioned at the space focus

$x_{SF} = 2x_A$

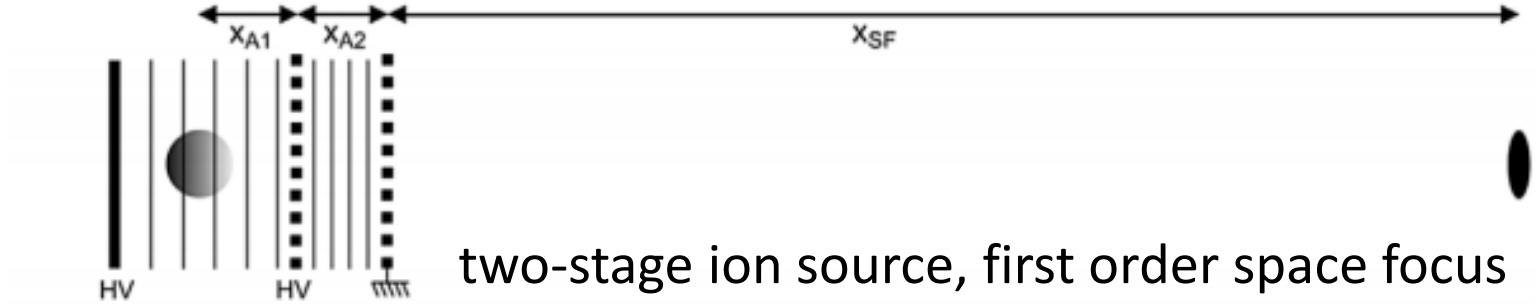
Very short!




single stage ion source, first order space focus

$U = U_{A1} + U_{A2}$

adjustable




two-stage ion source, first order space focus

$x_{SF}$ is tunable!


two-stage ion source, second order space focus

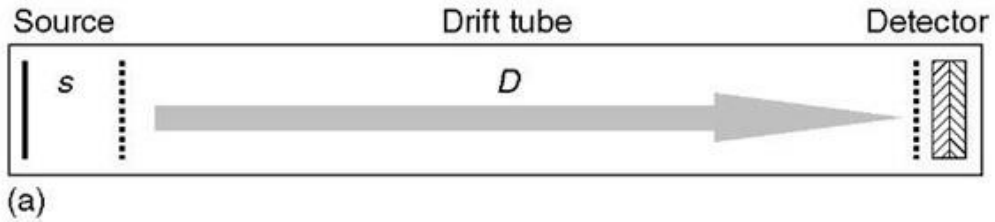
Boesi U. Mass Spectrom Rev 2017; 36:86.

# Problem is…

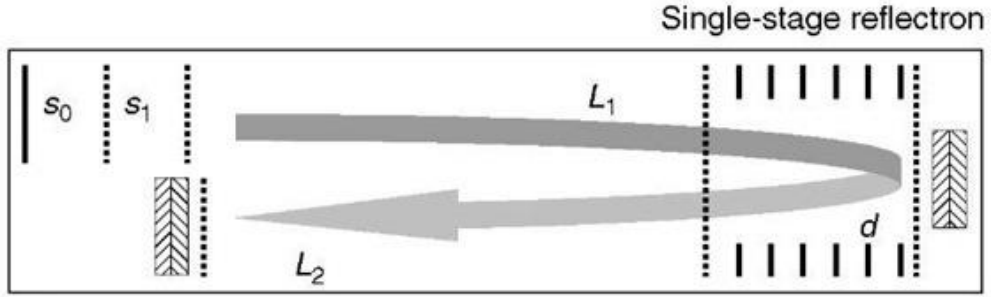
- high mass resolution requires long flight paths (large $x_{SF}$), in turn requiring a second order space focus

- second order space focus can be generated using a weak electric field (small $E_A$)

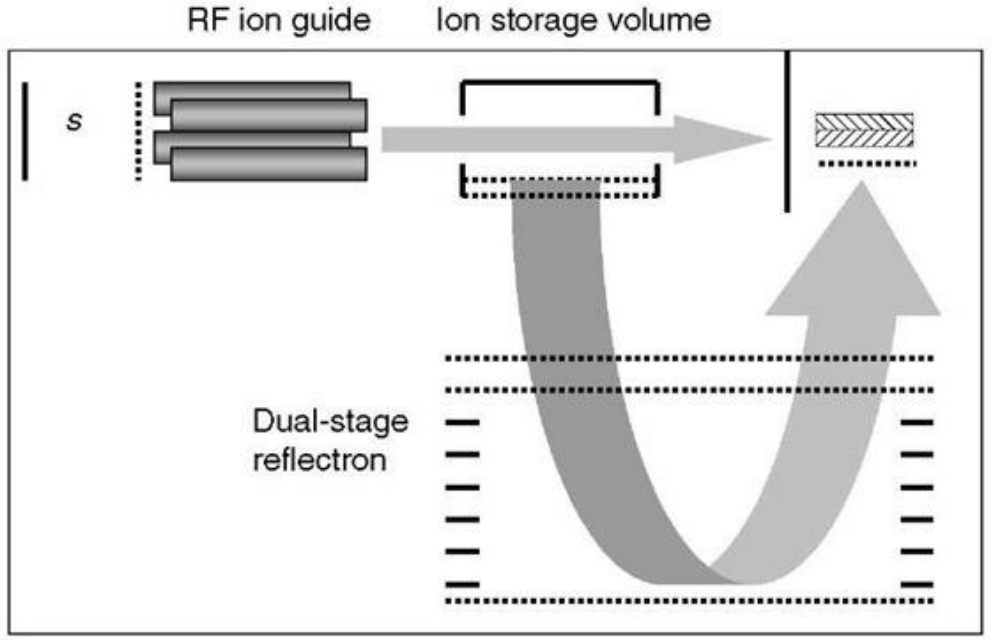
- compensation for $\Delta v$ requires a strong electric field (large $E_A$)




(a)




(b)

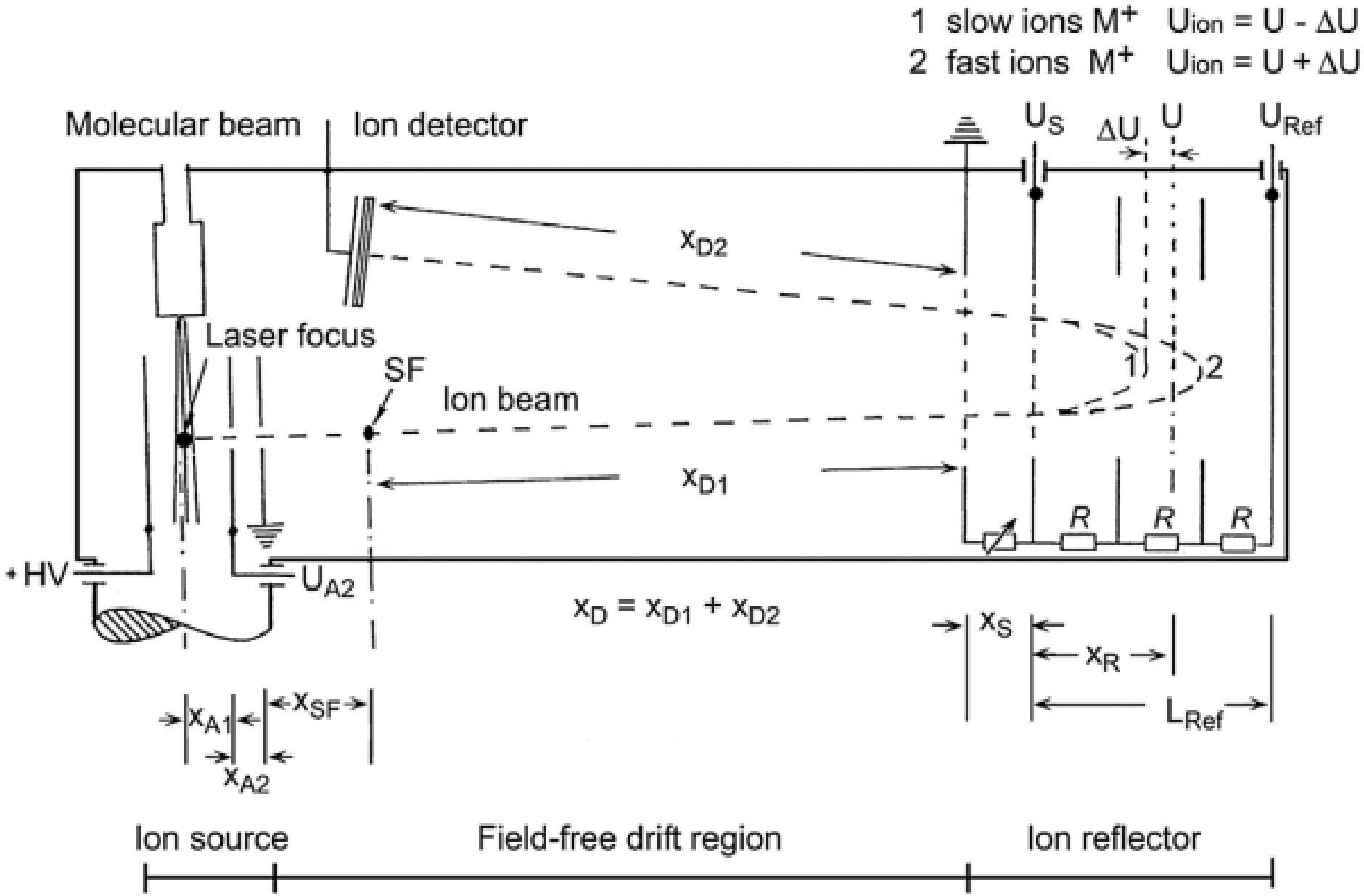



(c)

Linear TOF: limits mass resolution and mass range

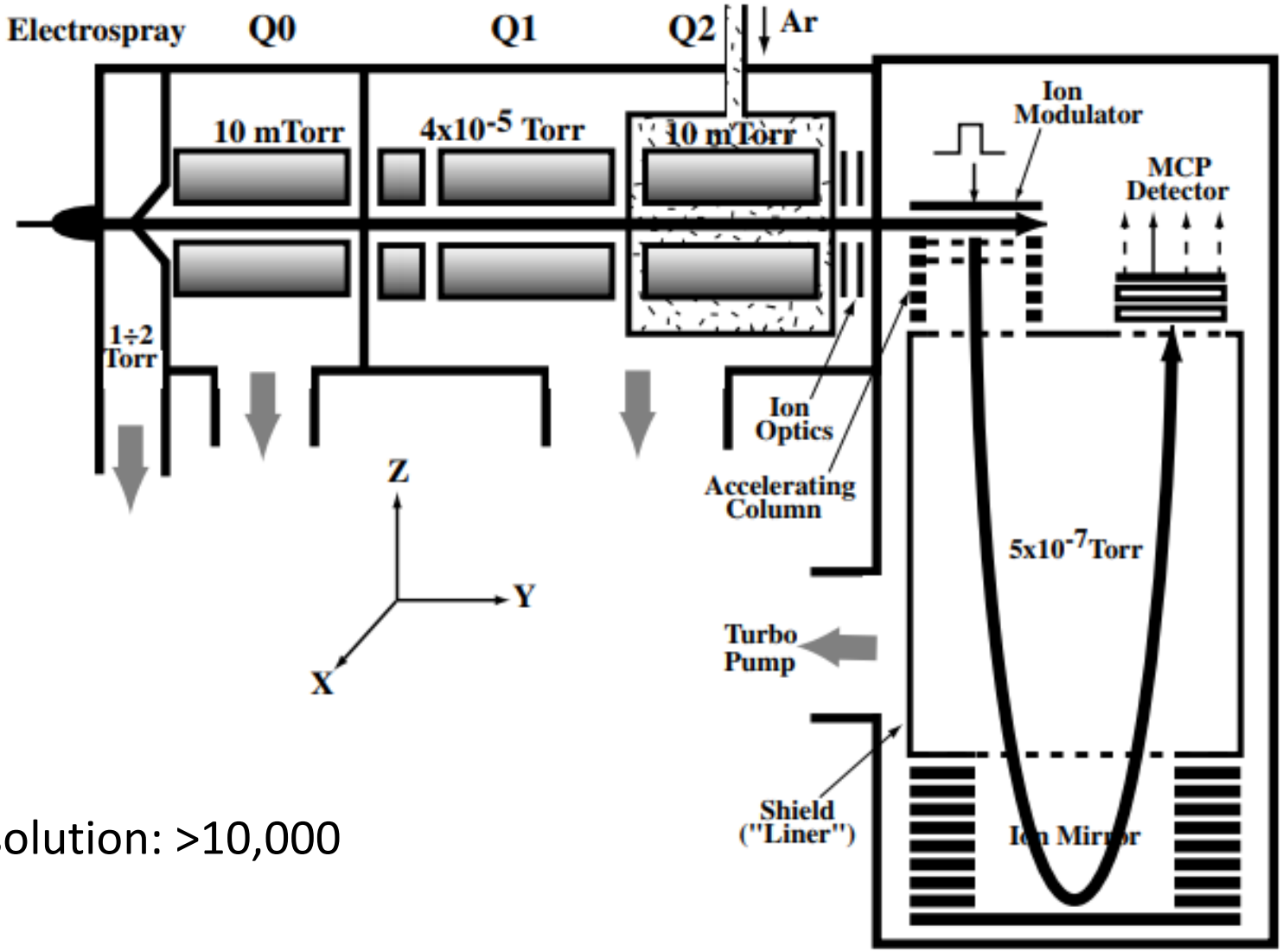
Single-stage reflectron TOF: linear potential throughout

Dual-stage reflectron TOF: (1) high electric field, (2) low electric field




Boesi U. Mass Spectrom Rev 2017; 36:86.

# Quadrupole-time-of-flight mass analyzers



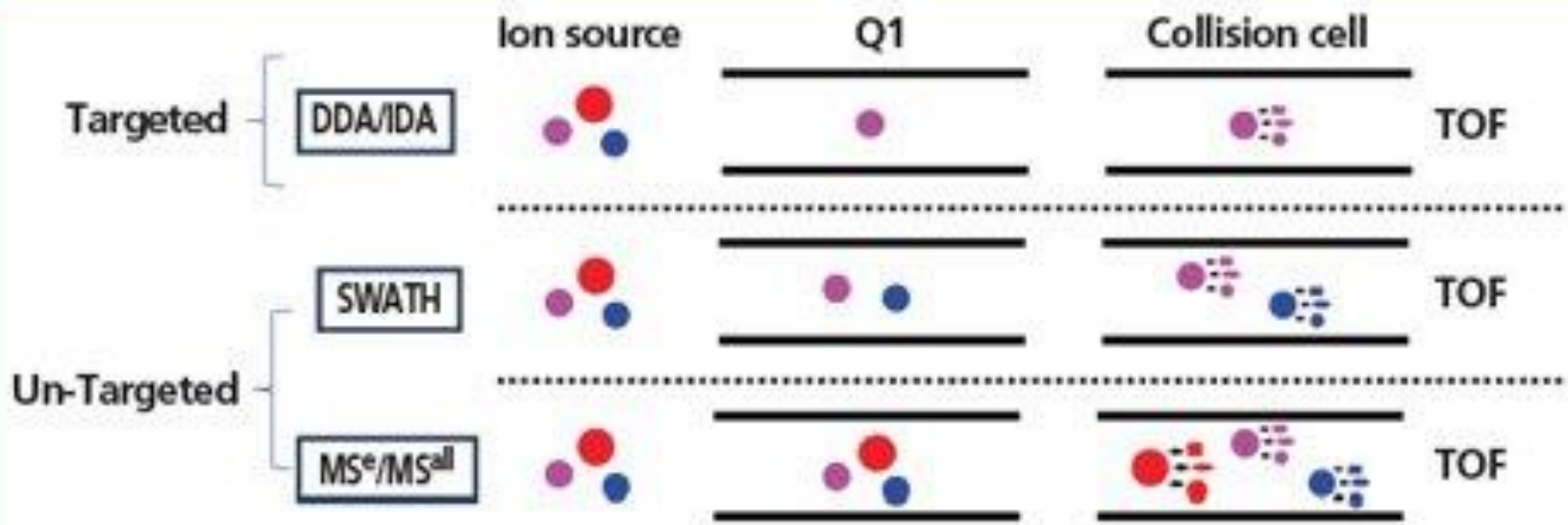

Resolution: >10,000

**Table 2. Key characteristics of QqLIT and QqTOF methods.**

| Characteristic | QqLIT | QqTOF |
|---|---|---|
| Analytical sensitivity | Better analytical sensitivity | Some analytical sensitivity is lost when collecting data in an untargeted manner |
| Compound identification | SRM methods are targeted in nature—information will be collected only on the presence or absence of compounds included in the acquisition method; compounds outside of this method cannot be identified | Untargeted data collection allows for the identification of compounds that are outside of the library or are unexpected |
| Method development | Compound-dependent parameters must be established for each compound in the acquisition method; the method must be updated when adding new compounds | Method development is simpler and faster; there is no need to develop compound-dependent parameters when collecting data in an untargeted manner; HRMS methods are more adaptable |
| Data analysis | Only targeted data analysis (i.e., library searching) can be performed | Targeted data analysis can be performed; the processing time and complexity are similar to the QqLIT method; other data analysis strategies (suspect and untargeted screening) are possible; these analyses can be more complex |
| Cost | Triple quadrupole and QqLIT instruments are less expensive than high-resolution instruments | HRMS instruments are more expensive |

Thoren KL et al. Clin Chem 2016;62:170.

**Figure 3:** An overiew of different quan–qual MS acquisition strategies. Information or Data Dependent Acquisition (IDA or DDA), Sequential Window Acquisition of all THeoretical fragment ion spectra (SWATH), MS/MS of all ions ($MS^{all}$ or $MS^{e}$). Adapted with permission from reference 8.



# Sample preparation for liquid chromatography and liquid chromatography-tandem mass spectrometry

- biological samples can rarely be introduced into (U)HPLC systems directly
- sample preparation is fundamental for efficient, reproducible and maintainable analysis


We offer three kinds of service:
GOOD - CHEAP - FAST
You can pick any two
GOOD service CHEAP won't be FAST
GOOD service FAST won't be CHEAP
FAST service CHEAP won't be GOOD

# Key issues in laboratories performing routine analytical tasks

- What laboratories?

- Quality of analytical information
- Maintenance costs
- Temporal optimization of workflows
- Integration of lab operation
- Safety and regulatory issues
- Efficient knowledge transfer


SOPs
Process Reviews
Monitoring Oversight (quality visits, document reviews)
Regulatory requirements & compliance oversight/ reviews
Audits and Inspections
Training
Quality Metrics
Quality Management

# Phases of the analytical service

- Preanalytical: sample collection, transport, storage and processing
- Analytical: peforming the assay
- Postanalytical: evaluating, validating, archiving and delivering results

**The quality of the analytical service is directly and strongly related to finding and eliminating/optimally reducing errors in our processes**

# Factors influencing quality

| Preanalytical | Analytical | Postanalytical |
|---|---|---|
| Sample collection: | Performance of analytical equipment | Methodology of data evaluation |
| Subject identification | Personnel | Data interpretation |
| Collection methodology | Quality of reagents | Validation of assay results |
| Selection of the sample container | Lab environment | Quality of result archive |
| Labeling the sample container | Methodology: | Deliver of results |
| Sample quantity | calibration model | |
| Sample protection | instrument settings | |
| Sample transport and storage | acceptance criteria | |
| Sample preparation: | QC setup: | |
| workflow optimization | calibration | |
| quality of devices, consumables and reagents | internal and external quality control | |
| complexity of procedure | Recording analytical results | |

# So what's the deal?

- Preanalytical error: 50-70% of all errors
- Analytical error: <15% of all errors
- Postanalytical error: 15-50% of all errors

# The point of sample preparation is:

- To obtain a sample that can be assayed **efficiently** → depends on the analyte, the sample matrix, the analytical instrumentation and the range of available sample prep devices

- Protect the analytical equipment in order to maintain its performance and the long-term quality of analytical results

- Reduce exposure to certain preanalytical errors

# Arsenal of sample preparation techniques:

- **physical:**
  - adsorption
  - centrifugation
  - dialysis
  - filtration
  - homogenization
  - incubation
  - mixing
  - sample concentration
  - sonication
- **chemical:**
  - digestion
  - dilution
  - distillation
  - extraction
  - homogenization
  - pH adjustment
  - protein precipitation
  - reactions (derivatization, oxidation/reduction etc.)
  - solvent exchange

# Types of analytes in biological specimens

- inorganic atoms, molecules and ions
- small organic molecules (MW<2000)
- large organic molecules (oligopeptides, proteins, nucleic acids, carbohydrates)

# Types of biological specimens

- gases (blood, exhaled, GI)
- fluids: blood, urine, saliva, tear, sweat, ascites, pus, synovial, dialysate, cell lysate etc.
- solids: hair, tissue homogenates, biopsy, skin peels, faeces, cells etc.

# Sample preparation for assaying small organic molecules

- Common approaches:
    - chemical derivatization
    - liquid-liquid extraction
    - pH adjustment prior to extraction or derivatization
    - phospholipid removal
    - protein precipitation
    - sample dilution
    - solid phase extraction
    - solvent exchange

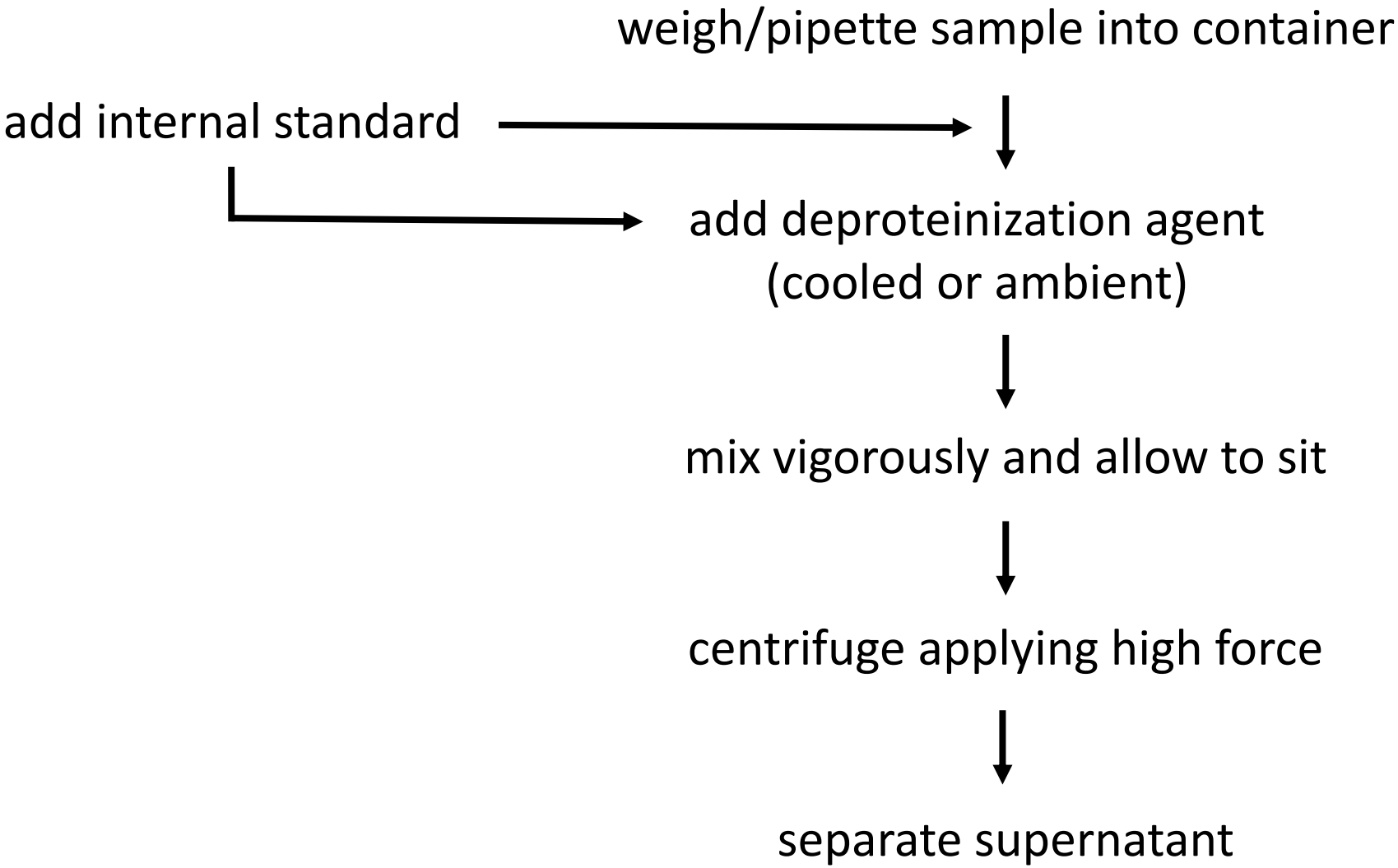
# sample deproteinization

- aims:
  - to obtain a sample ready for assaying
  - sample clean-up: protection of HPLC and MS, improvement of extraction recoveries, promotion of extraction procedure by reducing viscosity
  - recovery of protein-bound analyte fractions

- dilution of sample using solvents or solutions of inorganic salts

- commonly used deproteinizing agents:
  - organic solvents: acetonitrile, methanol, trifluoroacetic acid, trichloroacetic acid,
  - inorganic compounds: $HClO_4$, KOH, NaOH, $ZnSO_4$

# deproteinization workflow



# Factors in selecting the deproteinization agent

- type of sample
- type of analyte
- concentration range of analyte
- analytical aspects (instrumentation, chromatographic settings, injected volume)

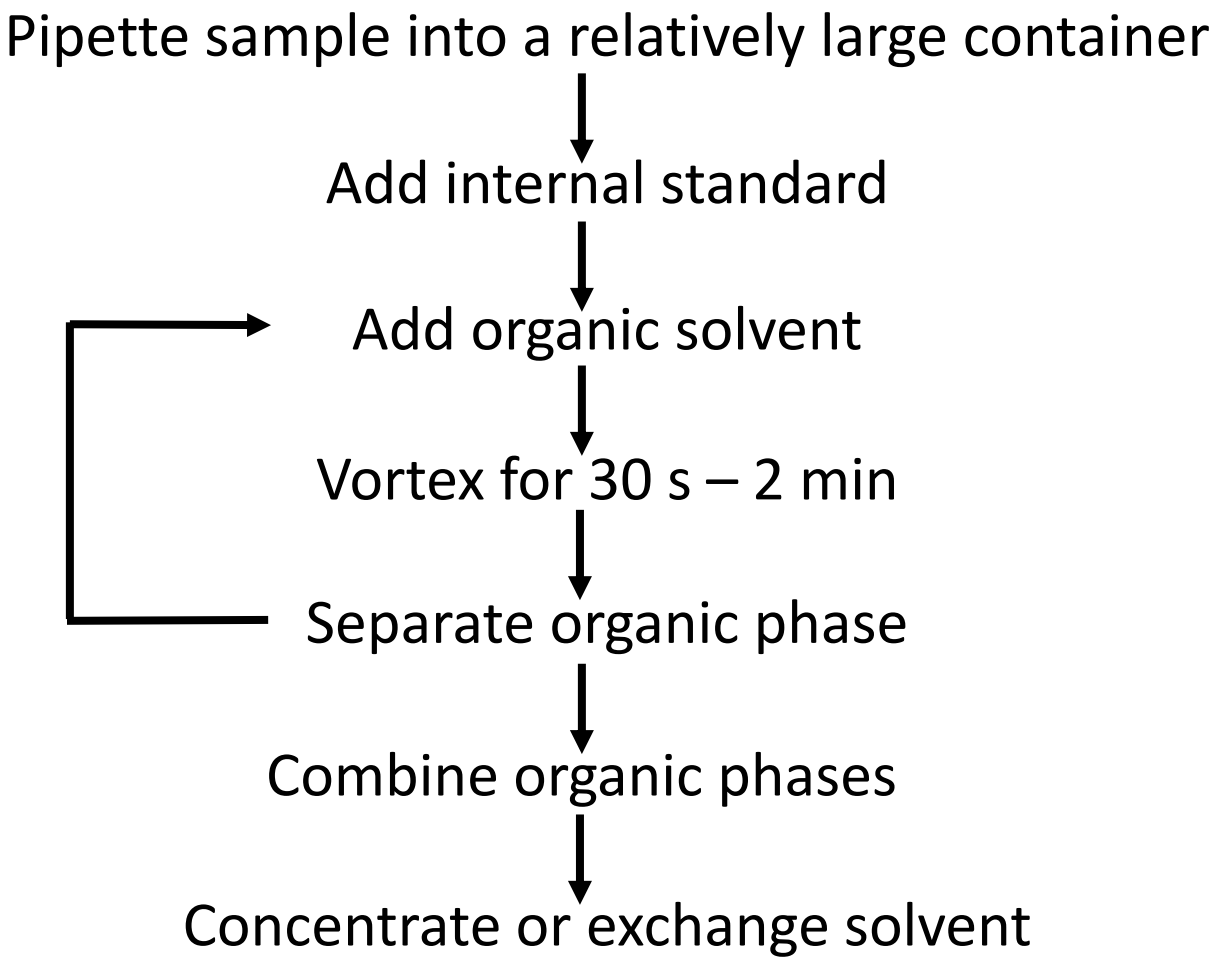
# Liquid-liquid extraction

- extraction of analytes from aqueous matrix into organic

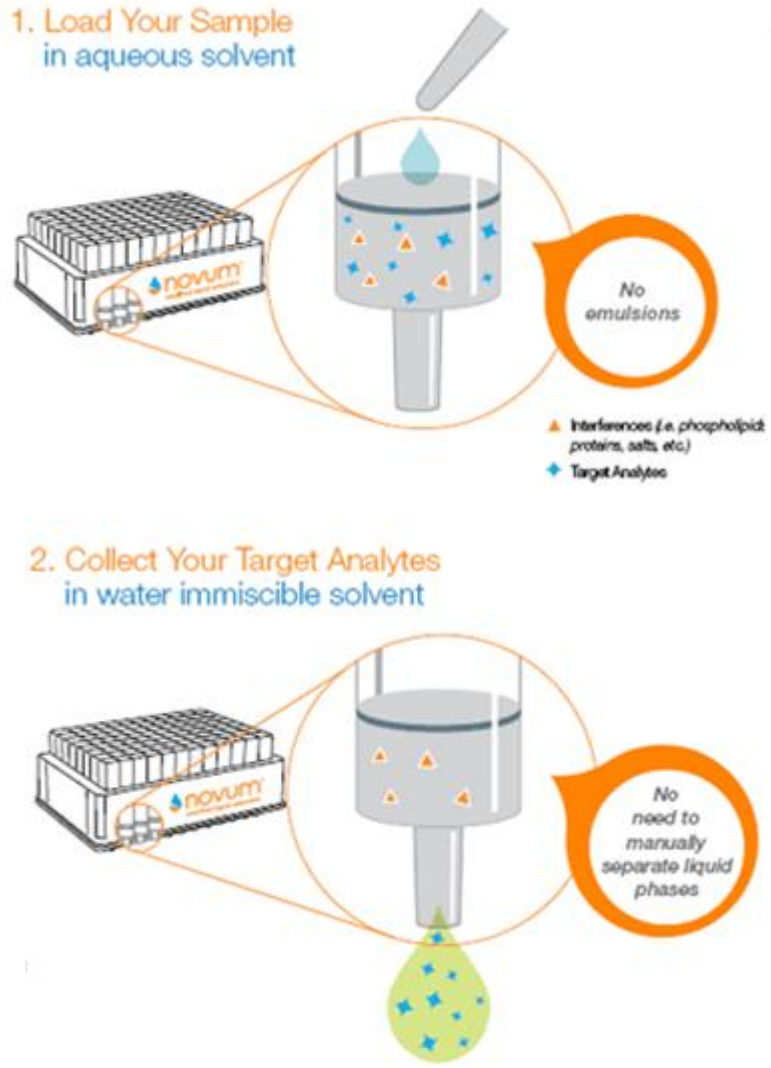
- advantages:
  - cheap
  - low matrix effect in MS analysis

- disadvantages:
  - laborious
  - difficult to increase throughput → simplified liquid extraction

# Liquid-liquid extraction workflow



# Simplified liquid extraction




Add internal standard to aliquoted sample

↓

Dilute sample using appropriate buffer
(render analytes in non-ionized form)

↓

Load sample, wait

↓

Elute

↓

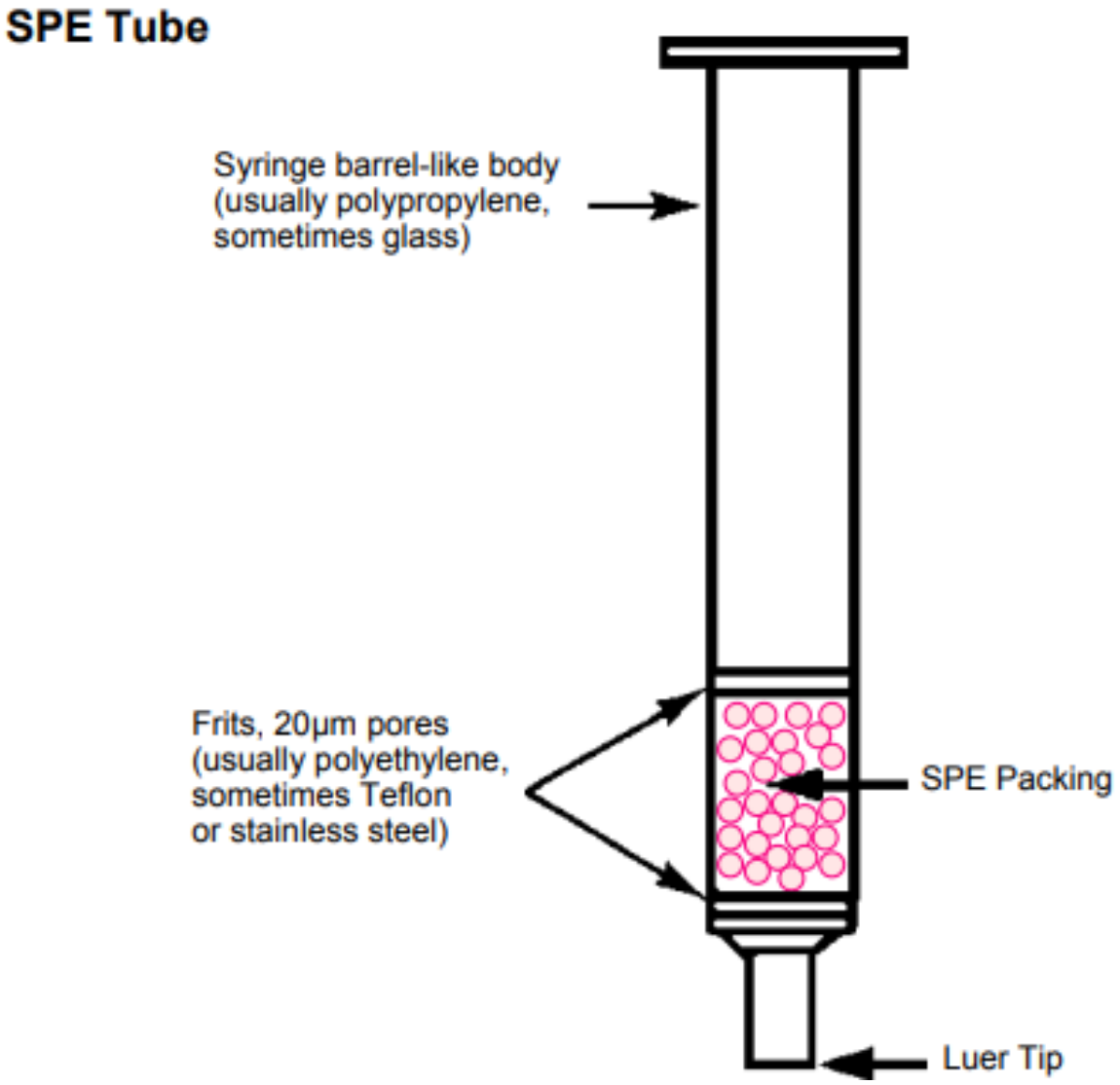
Concentrate or exchange solvent

# Analysis of vitamin D metabolites in plasma

- take 0.5 mL plasma
- add 10 µL IS solution (2.5 µg/mL D6-25-hydroxycholecalciferol in methanol)
- add 0.5 mL water
- extract 2 times using 1 mL ethyl acetate
- combine organic phases
- evaporate to dryness
- reconstitute using 0.5 mL methanol
- concentrate to 0.15 mL
- add 0.15 mL water

Karvaly G et al. Clin Chem Lab Med 2017;55:385.

# Solid phase extraction




Bulletin 910: Guide to solid phase extraction. Sigma Aldrich, 1998.

# The principles of solid phase extraction are very similar, but not analogous to liquid chromatography

- several HPLC phases are used
- particle diameter is much larger (app. 30-60 µm)
- extraction tubes are tightly packed

# Sorbents employed in solid phase extraction

- normal phase:
  - alumina-based
  - florisil
  - polymer-based
  - silica-based

- reversed phase:
  - DVB (styrene-divinylbenzene)
  - polymer-based
  - silica-based

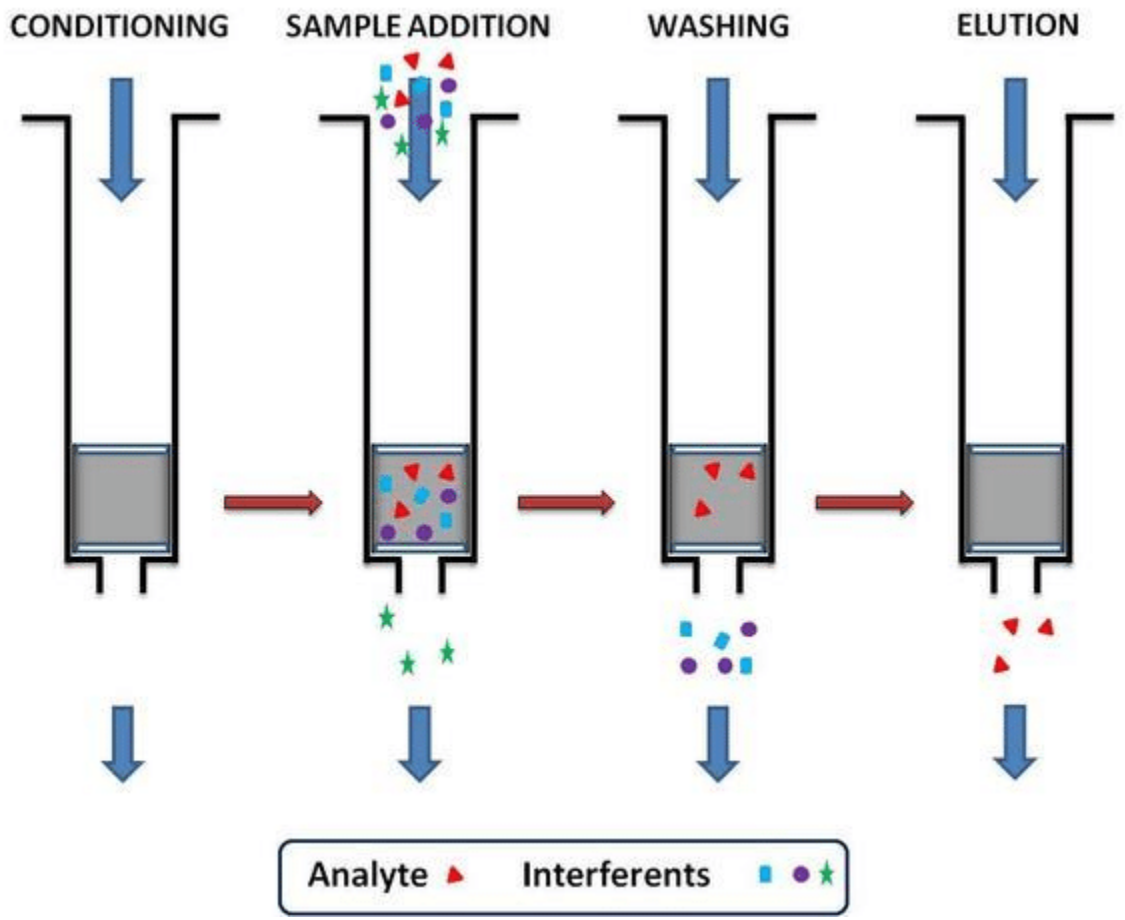
- ion exchange:
    - polymer-based

# Steps in developing an SPE method

- select a sorbent phase you expect to be suitable
- develop initial protocol: use analyte solution first
- compare to alternatives
- optimize variables using spiked matrix (solvents, pH, volumes, steps)
- check for ruggedness
- validate method

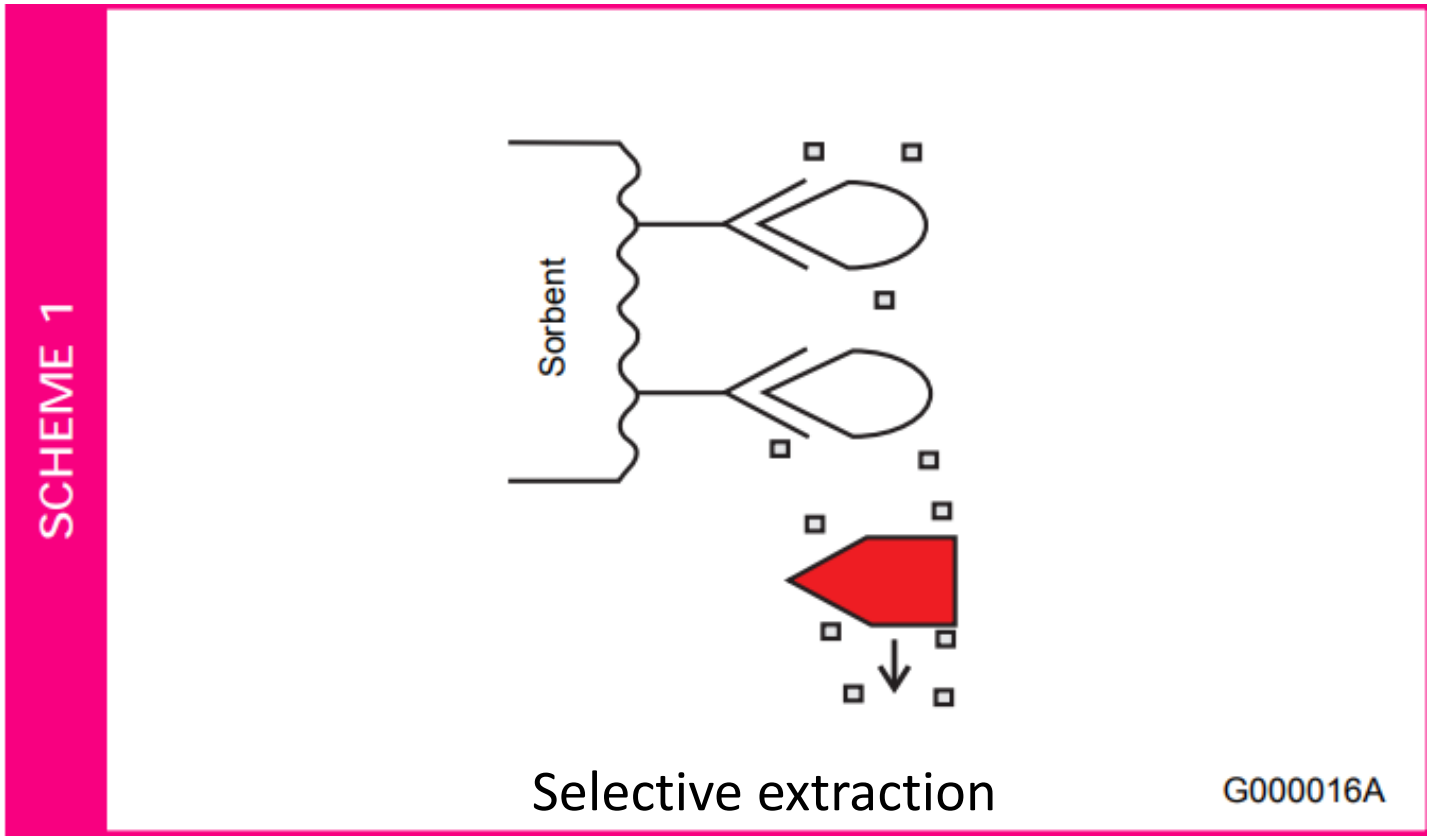
**Analyte recovery is important, but is only one variable among many!**

# SPE workflow




Bulletin 910: Guide to solid phase extraction. Sigma Aldrich, 1998.

# Uses of SPE

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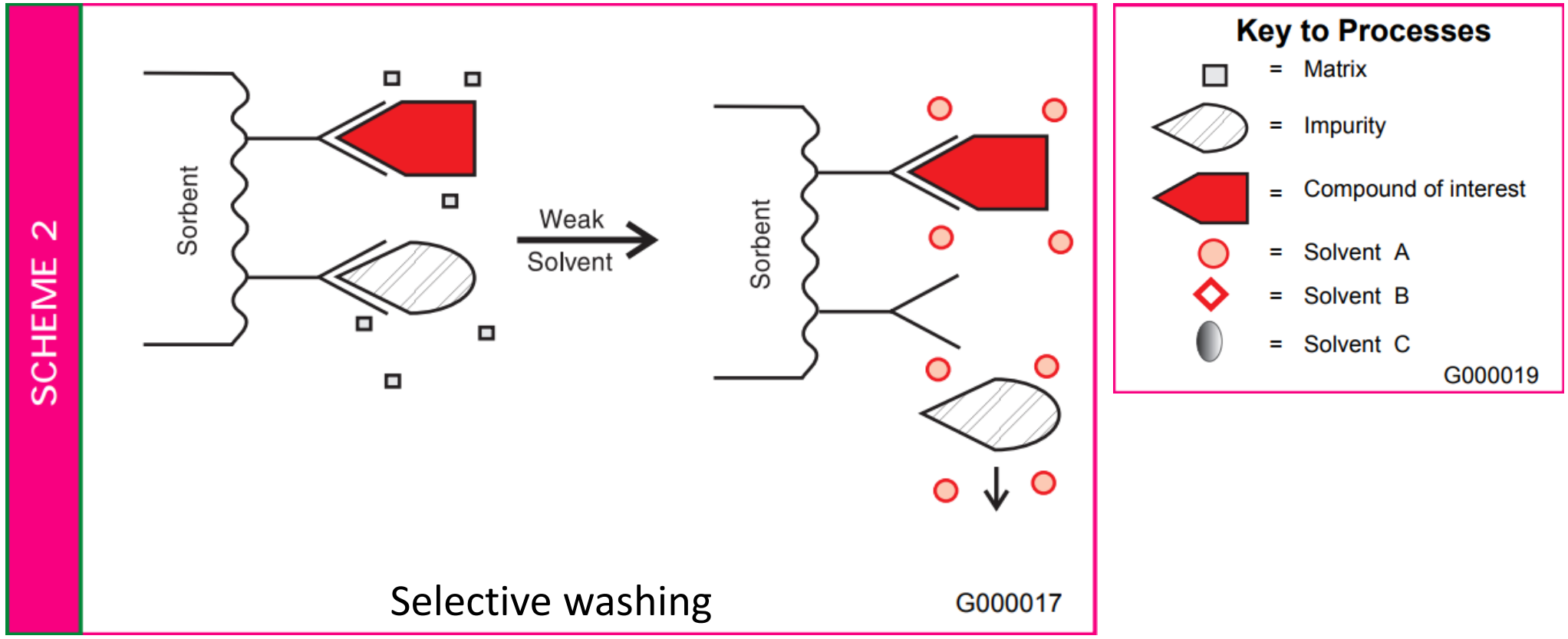


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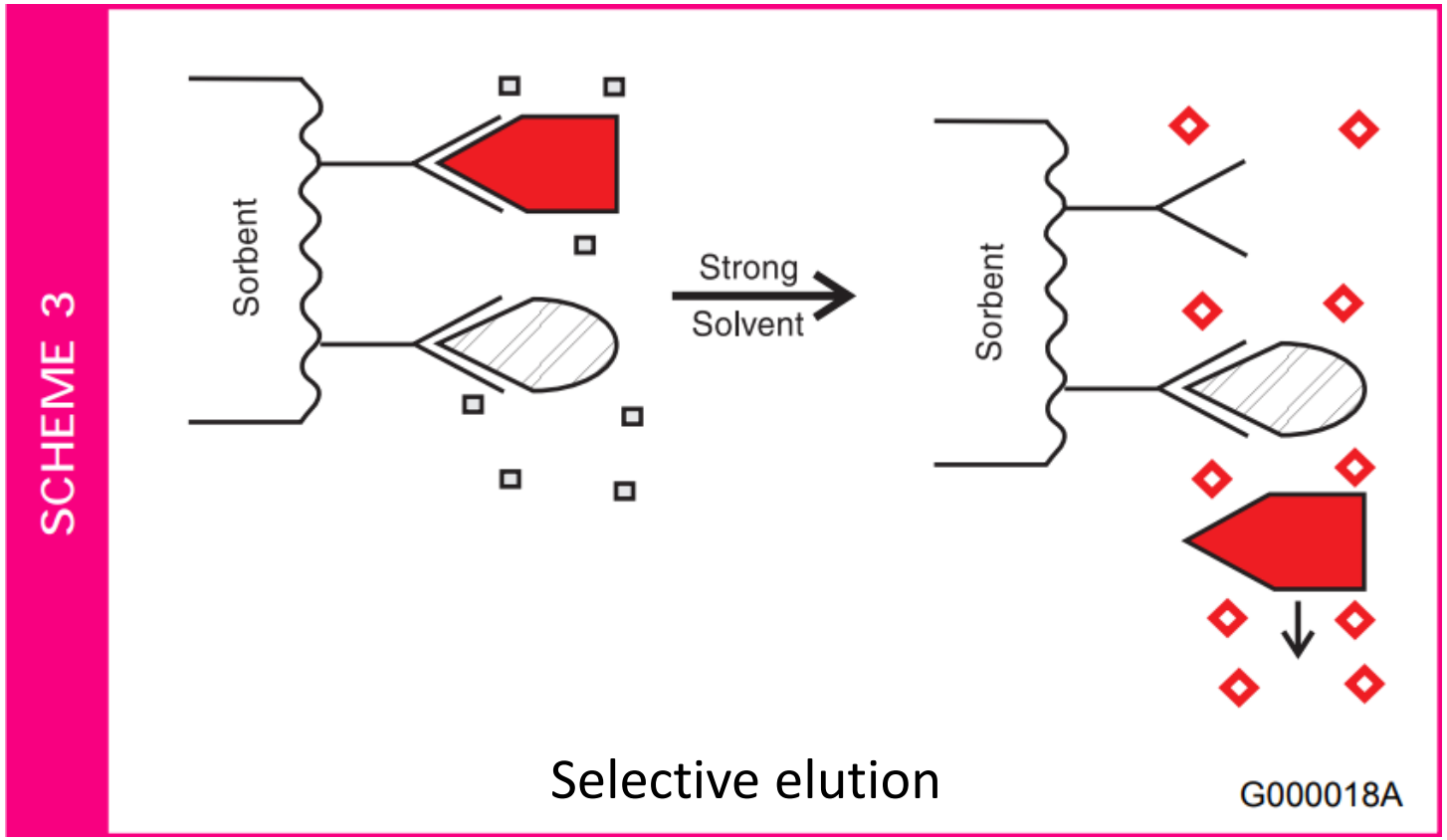
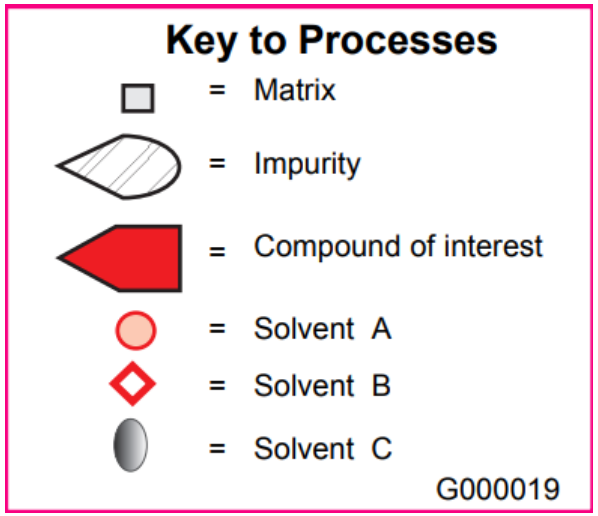



Bulletin 910: Guide to solid phase extraction. Sigma Aldrich, 1998.

# Mechanisms of retention on SPE phases

**Reversed Phase**
(polar liquid phase, nonpolar modified solid phase)

Hydrophobic interactions

- nonpolar-nonpolar interactions
- van der Waals or dispersion forces

**Normal Phase**
(nonpolar liquid phase, polar modified solid phase)

Hydrophilic interactions

- polar-polar interactions
- hydrogen bonding
- pi-pi interactions
- dipole-dipole interactions
- dipole-induced dipole interactions

**Ion Exchange**

Electrostatic attraction of charged group on compound to a charged group on the sorbent's surface

**Adsorption**
(interactions of compounds with unmodified materials)

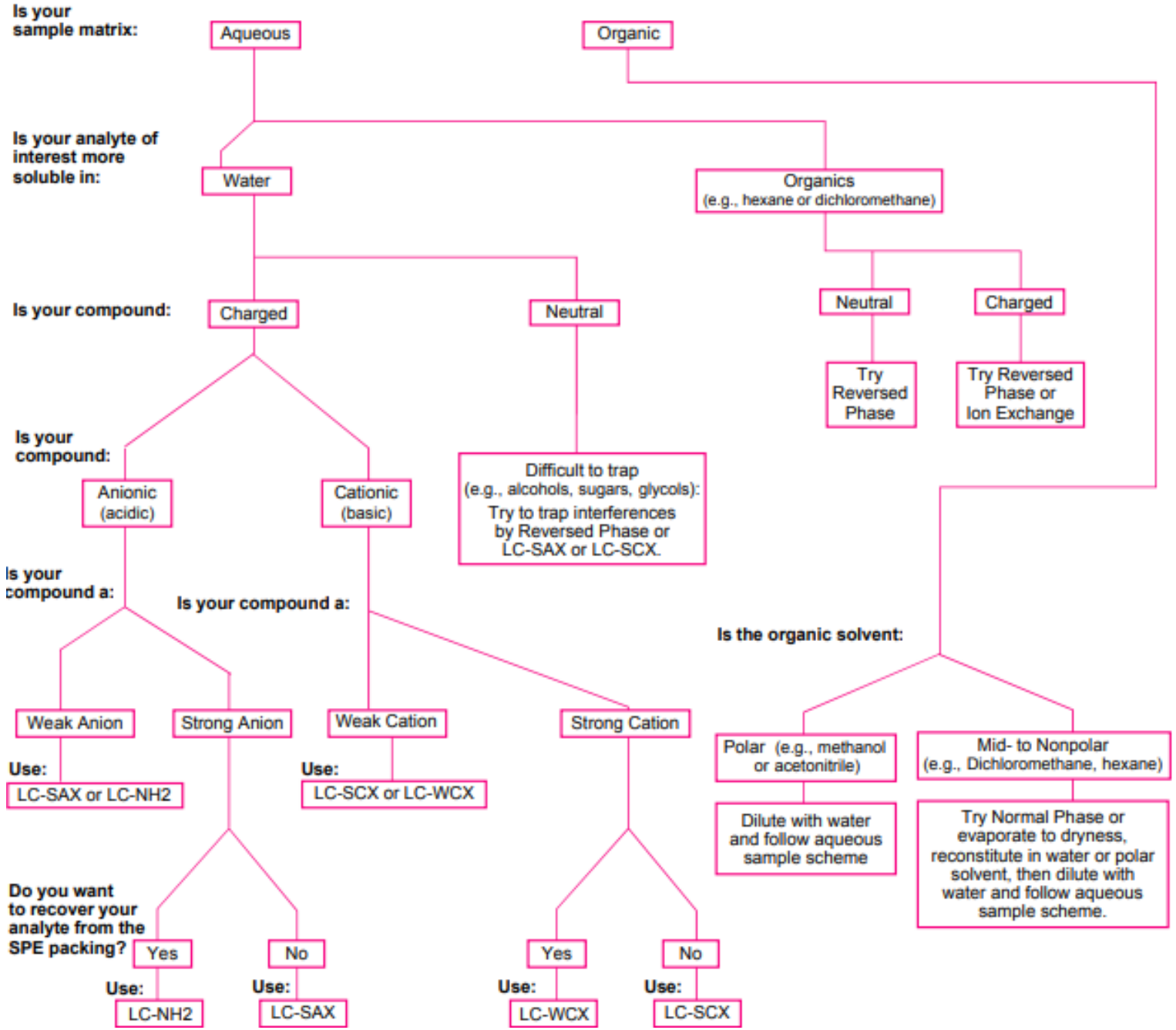
Hydrophobic and hydrophilic interactions may apply
Depends on which solid phase is used

Bulletin 910: Guide to solid phase extraction. Sigma Aldrich, 1998.

# Suitable target analytes

- inorganic ions (SAX, SCX)
- acidic and basic substances ($NH_2$, WAX, WCX)
- neutral small molecules (reversed phase)
- large biomolecules (proteins, nucleic acids)

# Sample Characteristics Determine Your SPE Procedure



## Table A. Characteristics of Solvents Commonly Used in SPE

| Polarity | | | Solvent | Miscible in Water? |
|---|---|---|---|---|
| Nonpolar | Strong Reversed Phase | Weak Normal Phase | Hexane | No |
| | | | Isooctane | No |
| | | | Carbon tetrachloride | No |
| | | | Chloroform | No |
| | | | Methylene chloride (dichloromethane) | No |
| | | | Tetrahydrofuran | Yes |
| | | | Diethyl ether | No |
| | | | Ethyl acetate | Poorly |
| | | | Acetone | Yes |
| | | | Acetonitrile | Yes |
| | | | Isopropanol | Yes |
| | Weak Reversed Phase | Strong Normal Phase | Methanol | Yes |
| | | | Water | Yes |
| Polar | | | Acetic acid | Yes |

# SPE formats

- standalone tubes
- tabless tubes
- 96-well plates