Fitting Linear Quadrupoles and Round Flight Tubes into the Square Clinical Laboratory

The What, Where and Why of Clinical Mass Spectrometry

Frederick G. Strathmann

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Present

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Objectives

- Describe the most common types of mass spectrometry
- List the areas of clinical laboratory medicine where mass spectrometry is currently used
- Compare the utility of various mass spectrometry techniques for specific applications in laboratory medicine
- Discuss several unique challenges of clinical mass spectrometry

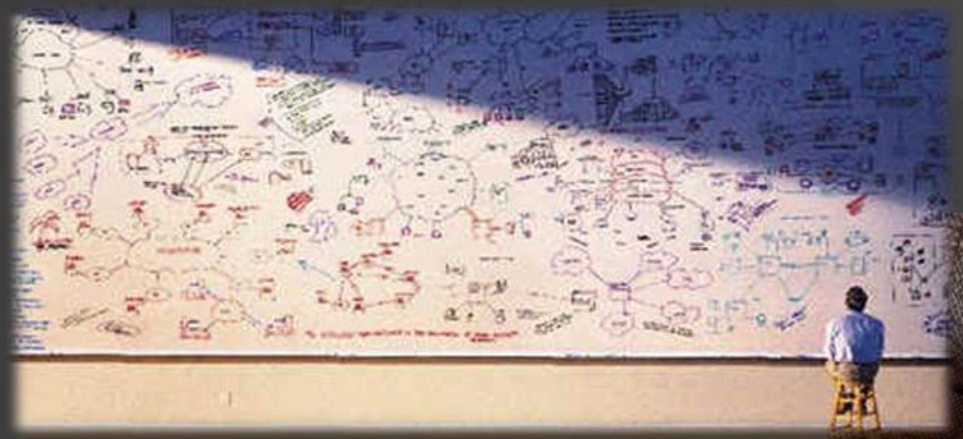
How to Get Mass Spectrometry into Your Laboratory

- What are you going to measure?
- What is the expected test volume?
- What will the TAT requirements be?
- What resources (money, people) do you have?

- *What technology is best?*

Where does Mass Spectrometry fit?

Toxicology



Endocrinology &
Biochemical Genetics

Microbiology

New Born Screening

Images: eofdreams.com; marketingsavant.com; momsguidetosandiego.com

Toxicology

The Comprehensive Drug Screen

- Gas Chromatography
- Single quadrupole mass spectrometry

Images: vias.org

Immunosuppressants

LC-MS/MS

- Tacrolimus
- Sirolimus
- Everolimus

- Cost effective*
- Increased throughput
- High specificity

Images: info.agscientific.com

Confirmation/Quantitation

LC-MS/MS a Gold Standard

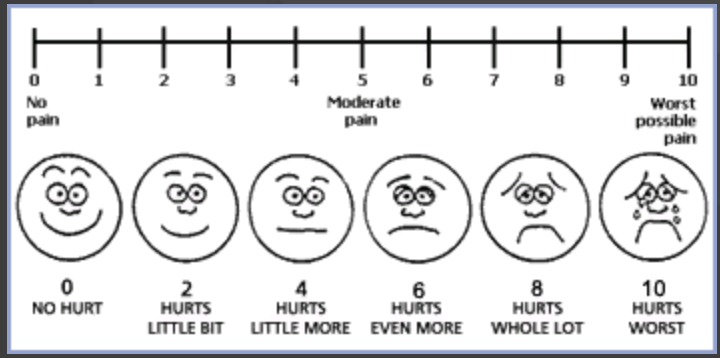
- Forensics
- Workplace testing
- Clinical laboratory?
  - Screen w/ “Reflex”

- Cost effective*
- Increased throughput
- High specificity

Pain Management

LC-MS/MS; GC-MS; LC-TOF

- Urine
- Qualitative vs. Quantitative
- Compliance Determination

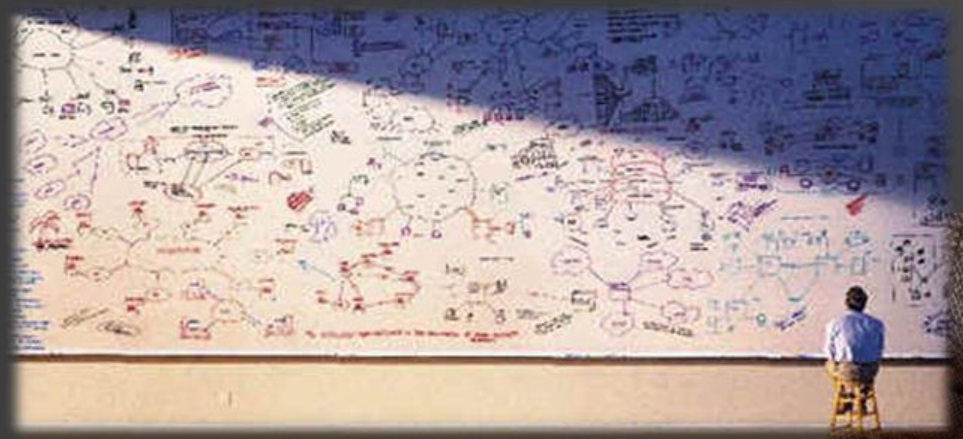


- Cost effective*
- Increased throughput
- High specificity

Images: smhc.org; aacc.org

Where does Mass Spectrometry fit?

Toxicology



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Vitamin D

- 25-OH vitamin D
  - C-3 Epimer
- 1,25-diOH vitamin D

- Cost effective*
- Increased throughput
- High specificity

Endogenous Steroids

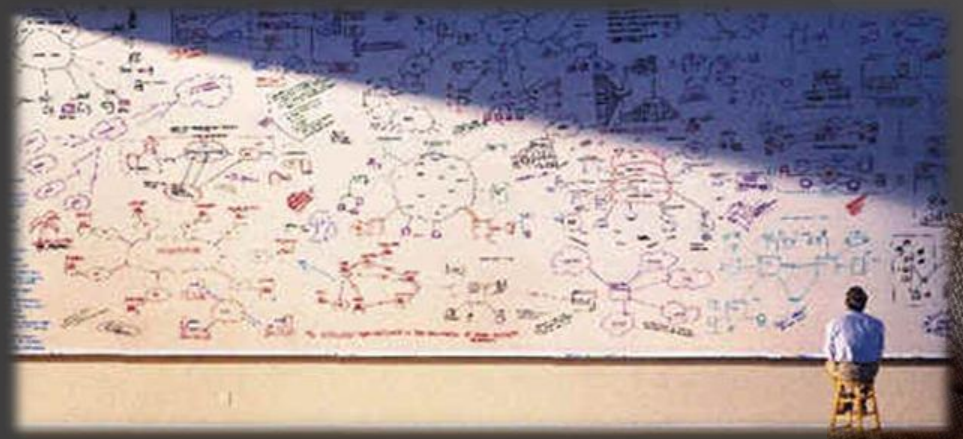
- Adrenal Steroids
- Glucocorticoids
- Androgens
- Estrogens

- Cost effective*
- Increased throughput
- High specificity

Images: webicina.com

Where does Mass Spectrometry fit?

Toxicology



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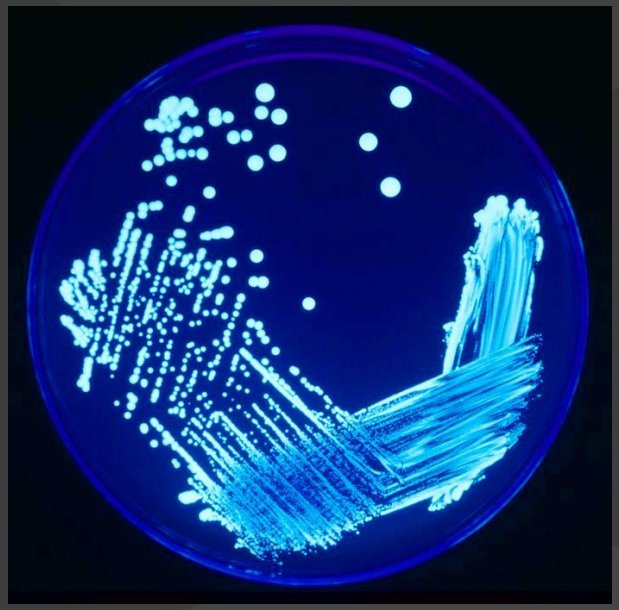
Microbiology

MALDI-TOF

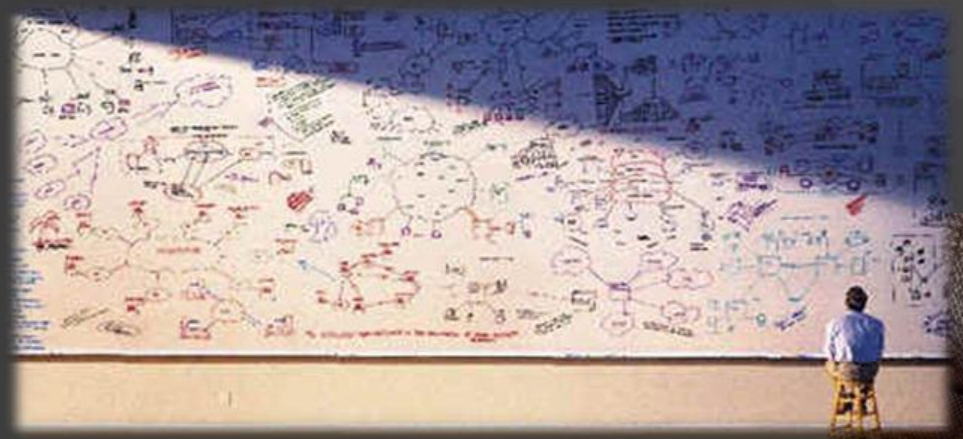
- Bacterial ID
- Fungal ID



- Cost effective*
- Increased throughput
- High specificity

Where does Mass Spectrometry fit?

Toxicology



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Biochemical Genetics Newborn Screening

LC-MS/MS

- Inborn Errors of Metabolism
- Malabsorption
- Malnutrition

- Cost effective*
- Increased throughput
- High specificity

Images: health.gov.il

Key Points

- Many areas of laboratory medicine are benefiting from the specificity of mass spectrometry
- Each area is pushing the field of clinical mass spectrometry in unique ways

Image: clickypix.com

The Fundamental Question

- What type of mass spectrometry is best?

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- ~~What type of mass spectrometry is best?~~
- What type of mass spectrometry is right for you?

How to Get Mass Spectrometry into Your Laboratory

- What are you going to measure?
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The Common Problems with All Mass Spectrometry

1. Biological specimens are mostly made of what we're not interested in measuring

2. Majority of clinical samples are liquid

3. Mass spectrometry only works with ions in the gas phase

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Sample Preparation Methods

Getting rid of the extra stuff

- Dilute and Shoot
- Protein crash
- Liquid-Liquid Extraction
- Supported Liquid Extraction
- Solid Phase Extraction

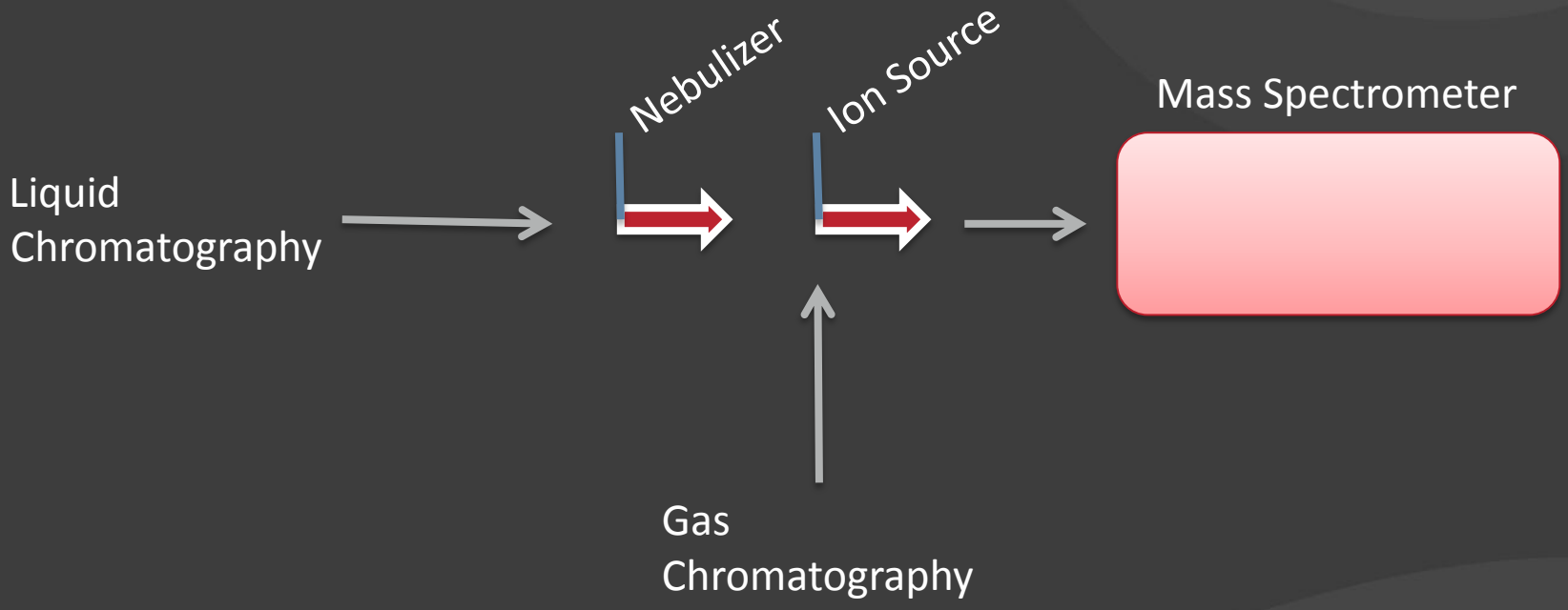
The Common Problems with All Mass Spectrometry

1. Biological specimens are mostly made of what we're not interested in measuring

2. Majority of samples are introduced as a liquid

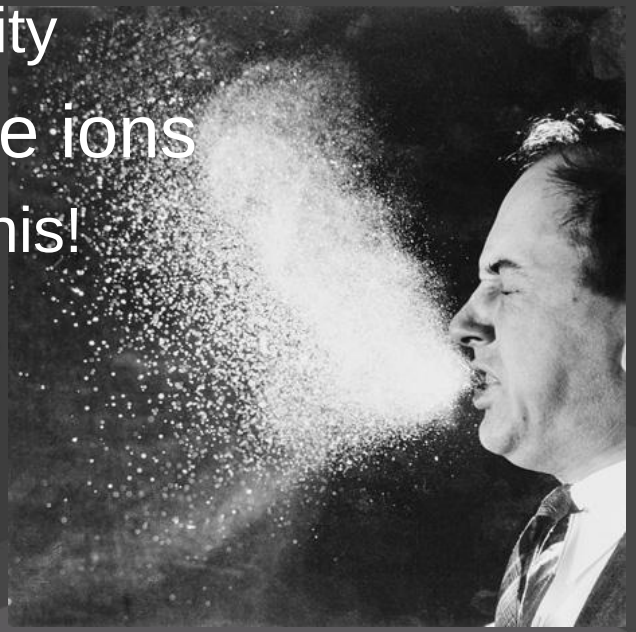
3. Mass spectrometry only works with ions in the gas phase

Key Steps Outline

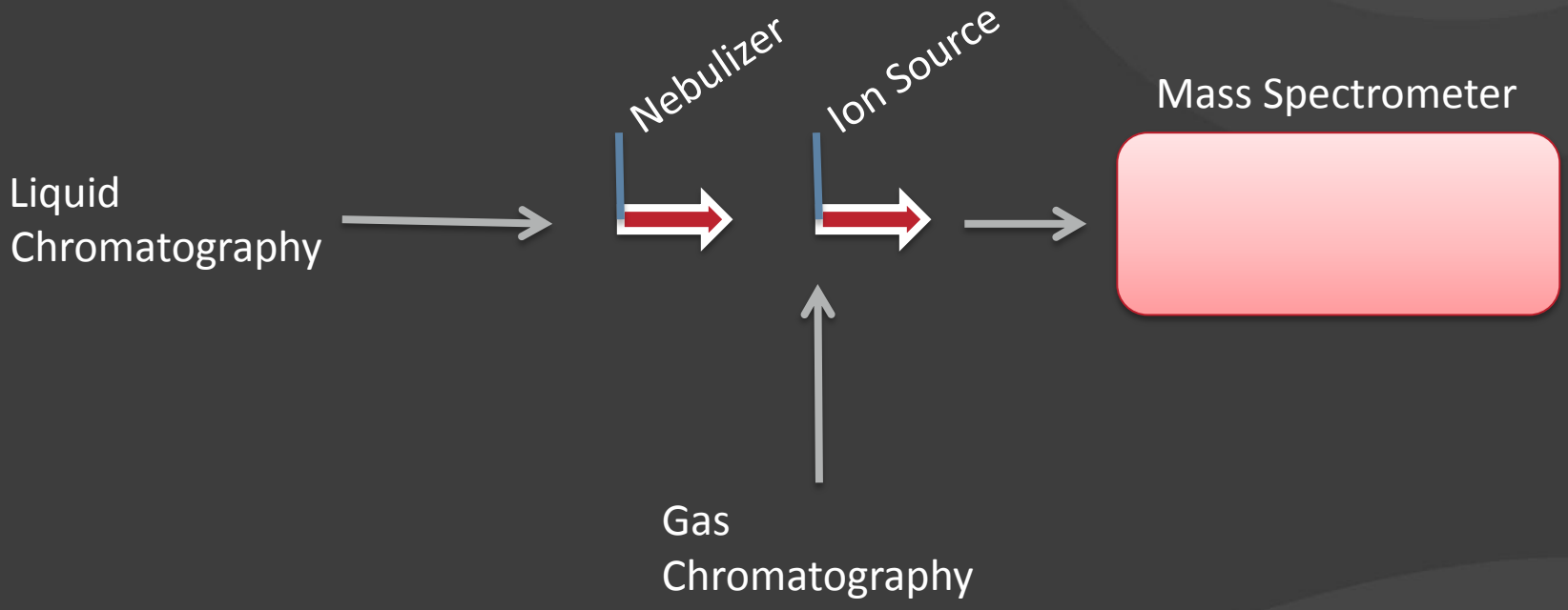


Key Steps in the Process

- Chromatographically separate the sample
  - Reduced complexity/noise
  - Improve the downstream data quality
- Turn liquid sample into gas phase ions
  - Gas chromatography solves ½ of this!
  - Nebulizer for liquid samples



Key Steps Outline

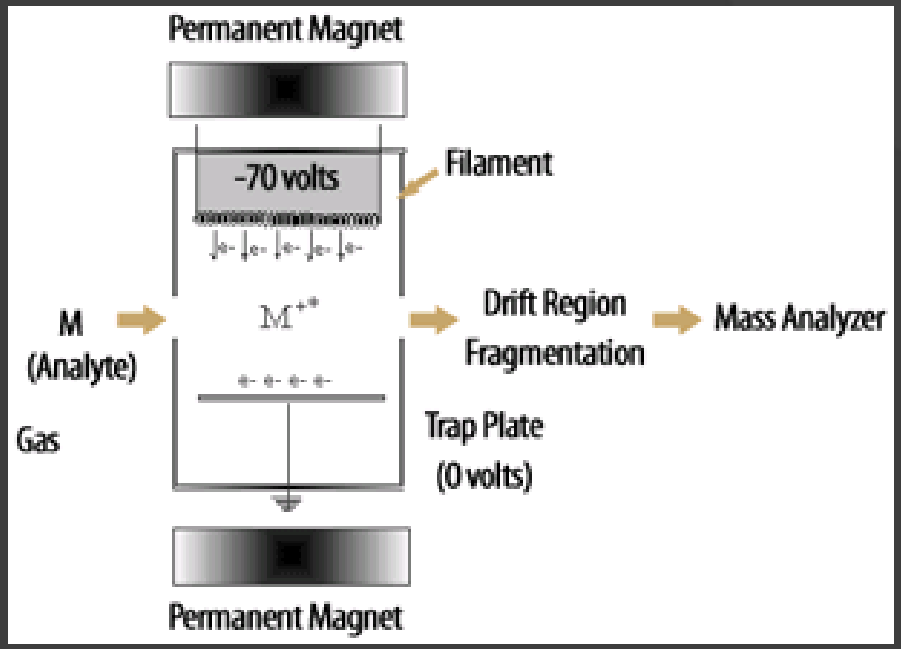


Ionization Sources

- Electron Bombardment
- Electrospray Ionization (ESI)
- Atmospheric Pressure Chemical Ionization (APCI)

Ionization Overview

Electron Bombardment



Images: noble.org

Ionization Overview

Electrospray

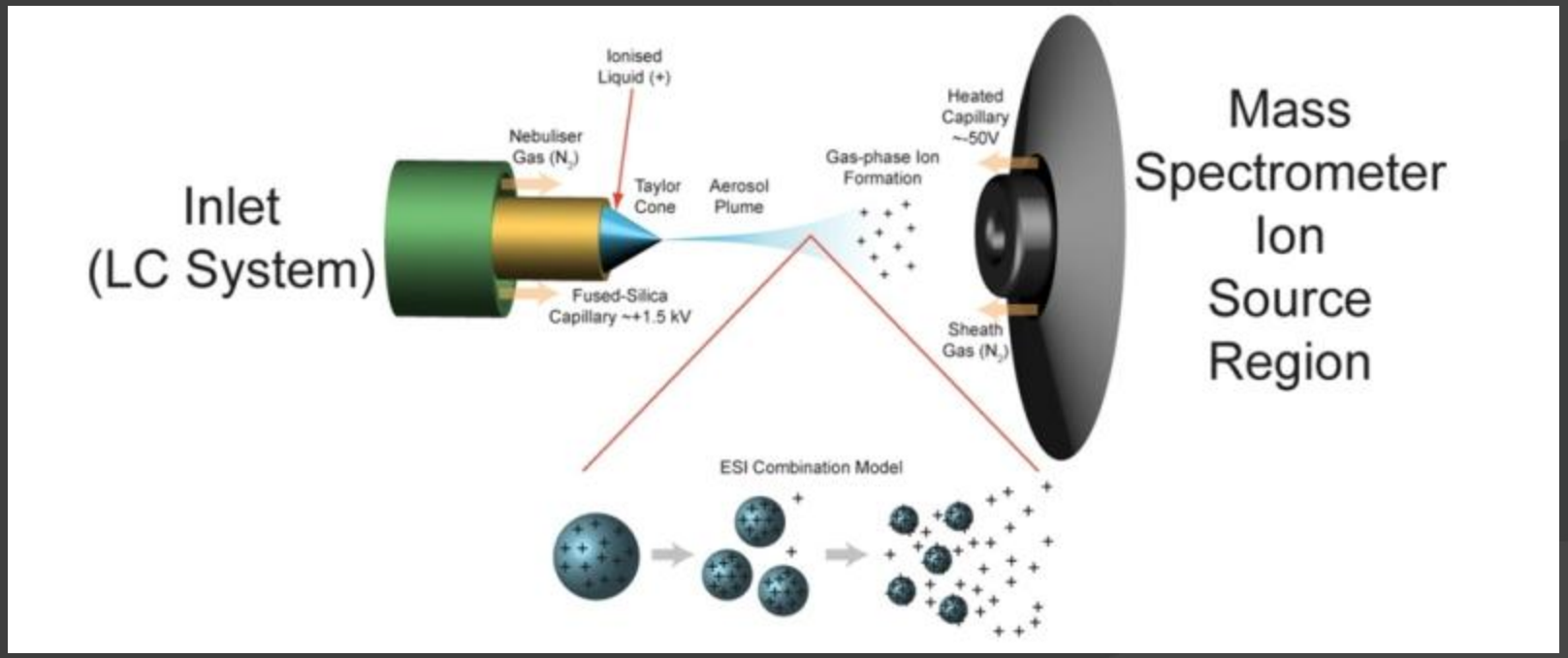
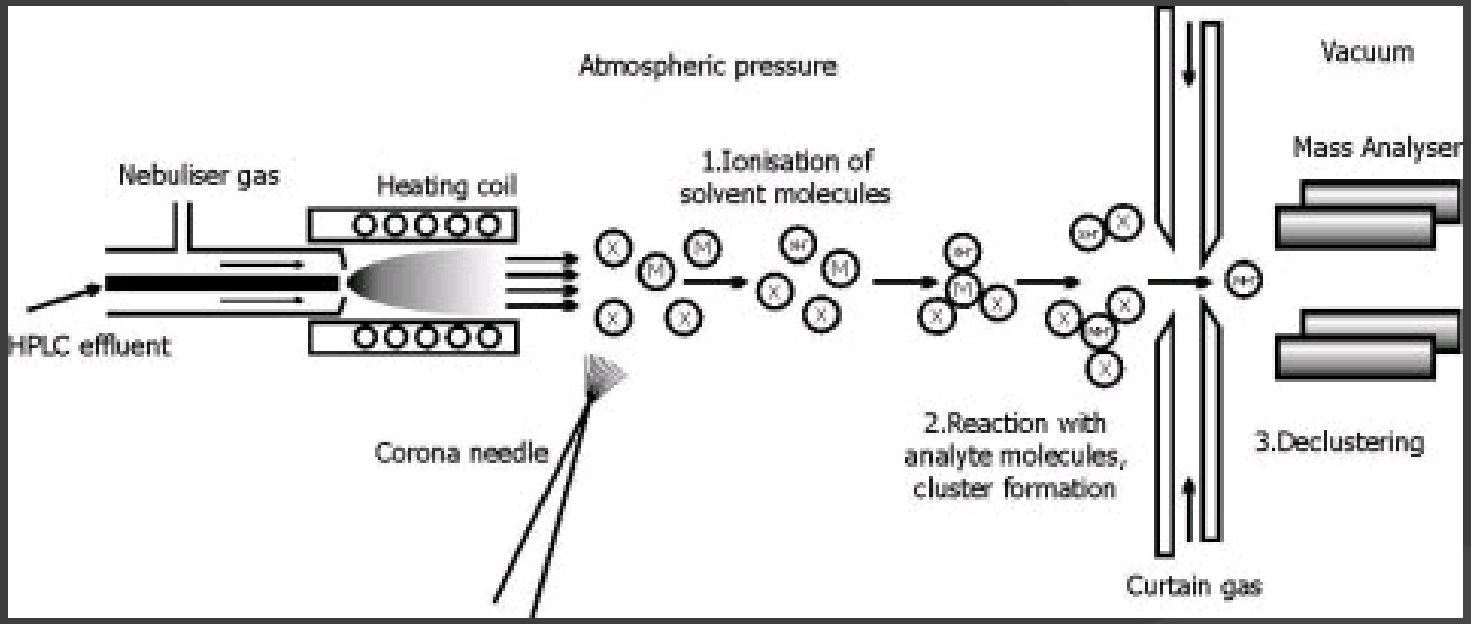


Image: lamondlab.com

Ionization Overview

Atmospheric Pressure Chemical

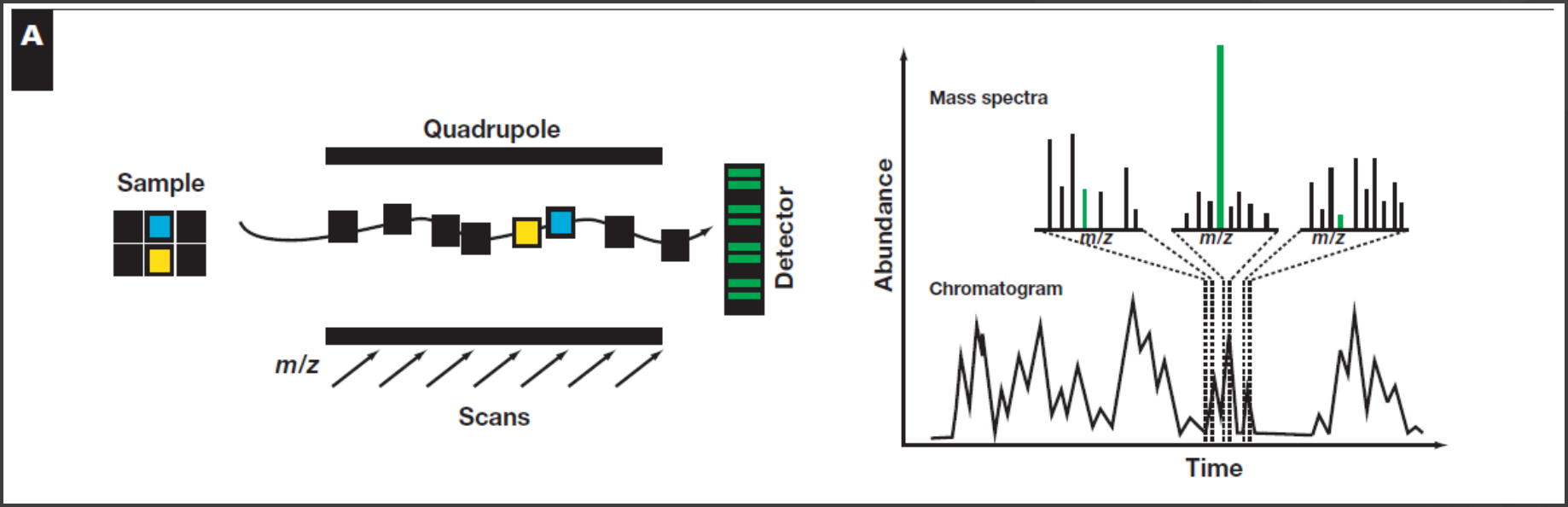


Images: services.leatherheadfood.com

Important Concepts

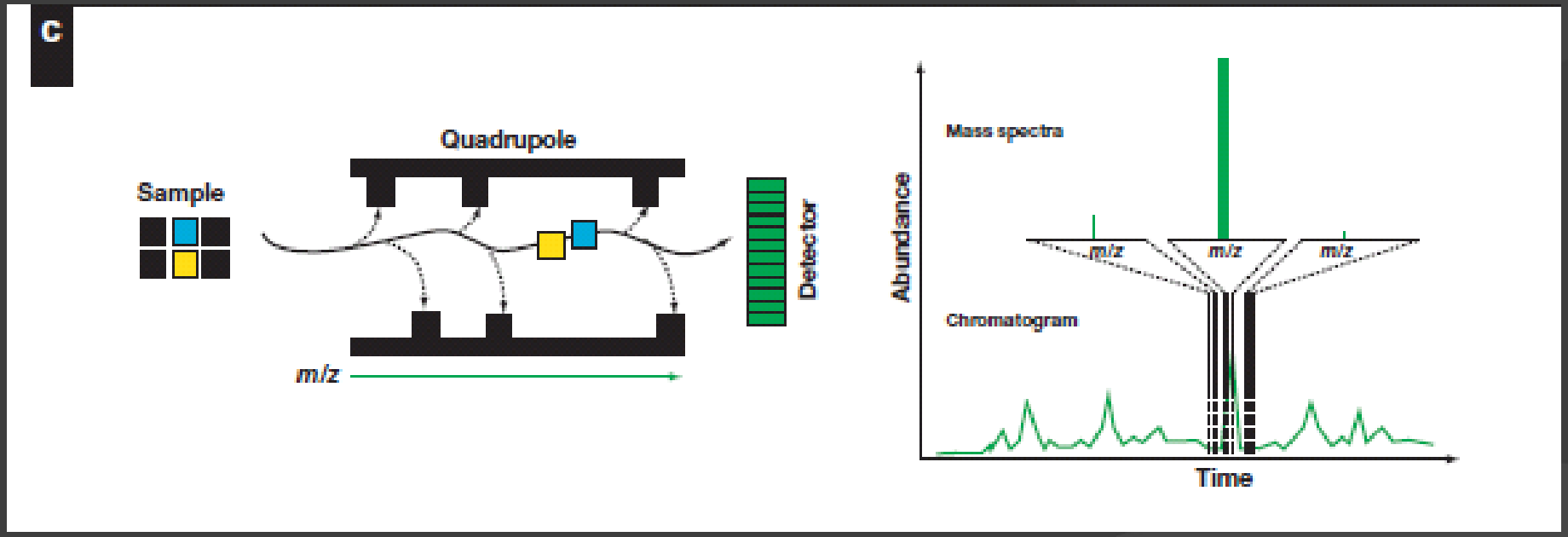
- A charged molecule (ion) can be manipulated using voltages
- “Steer” and “push” it in different directions

Single Quadrupole Mass Spectrometry



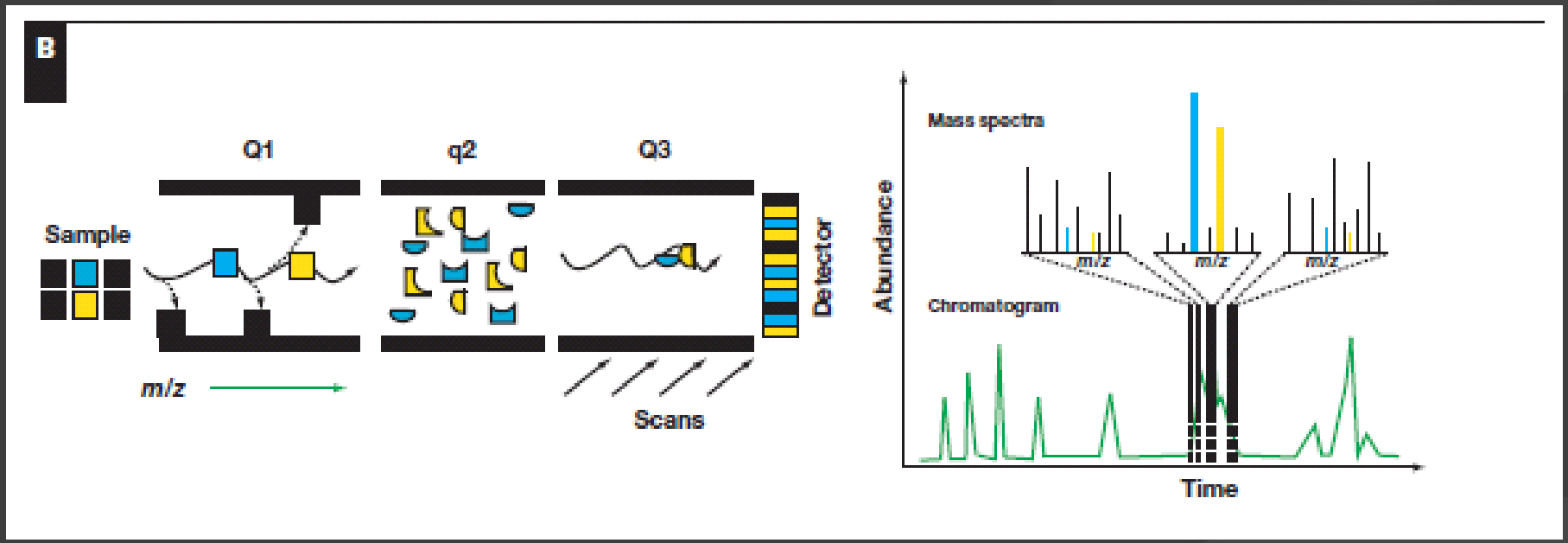
Strathmann, F. G. and A. N. Hoofnagle (2011). Am J Clin Pathol 136(4): 609-616.

Selected Ion Monitoring



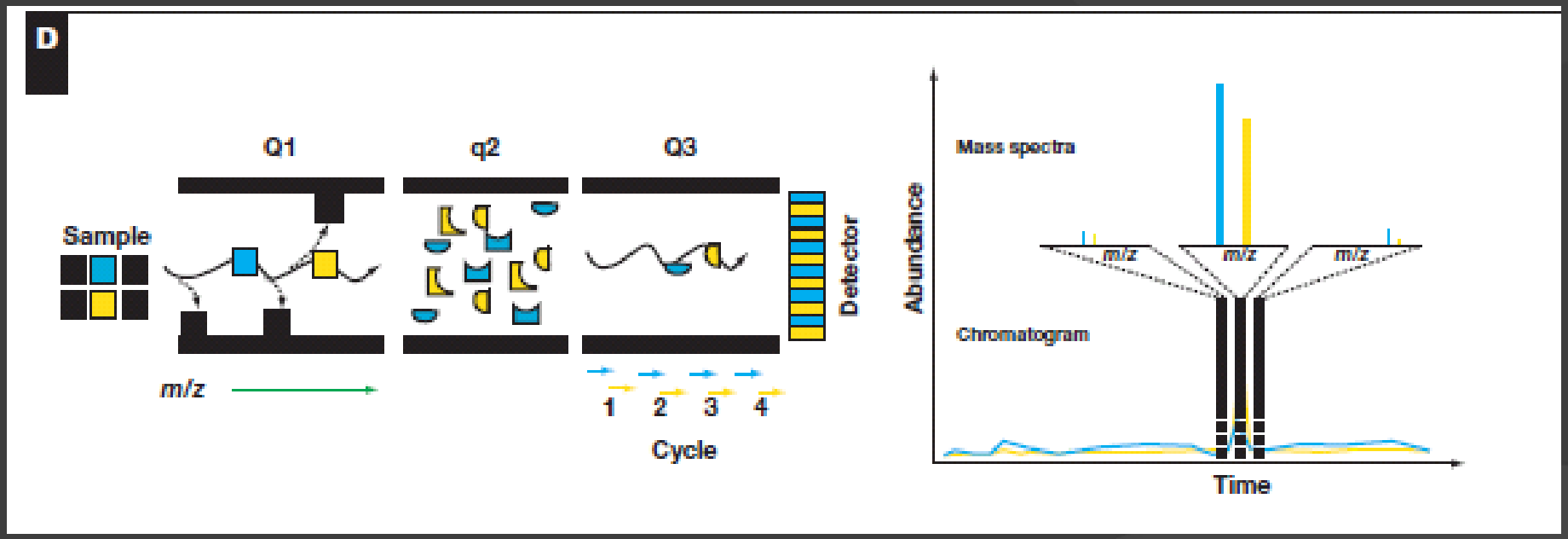
Strathmann, F. G. and A. N. Hoofnagle (2011). Am J Clin Pathol 136(4): 609-616.

Tandem Mass Spectrometry



Strathmann, F. G. and A. N. Hoofnagle (2011). Am J Clin Pathol 136(4): 609-616.

Multiple Reaction Monitoring



Strathmann, F. G. and A. N. Hoofnagle (2011). Am J Clin Pathol 136(4): 609-616.

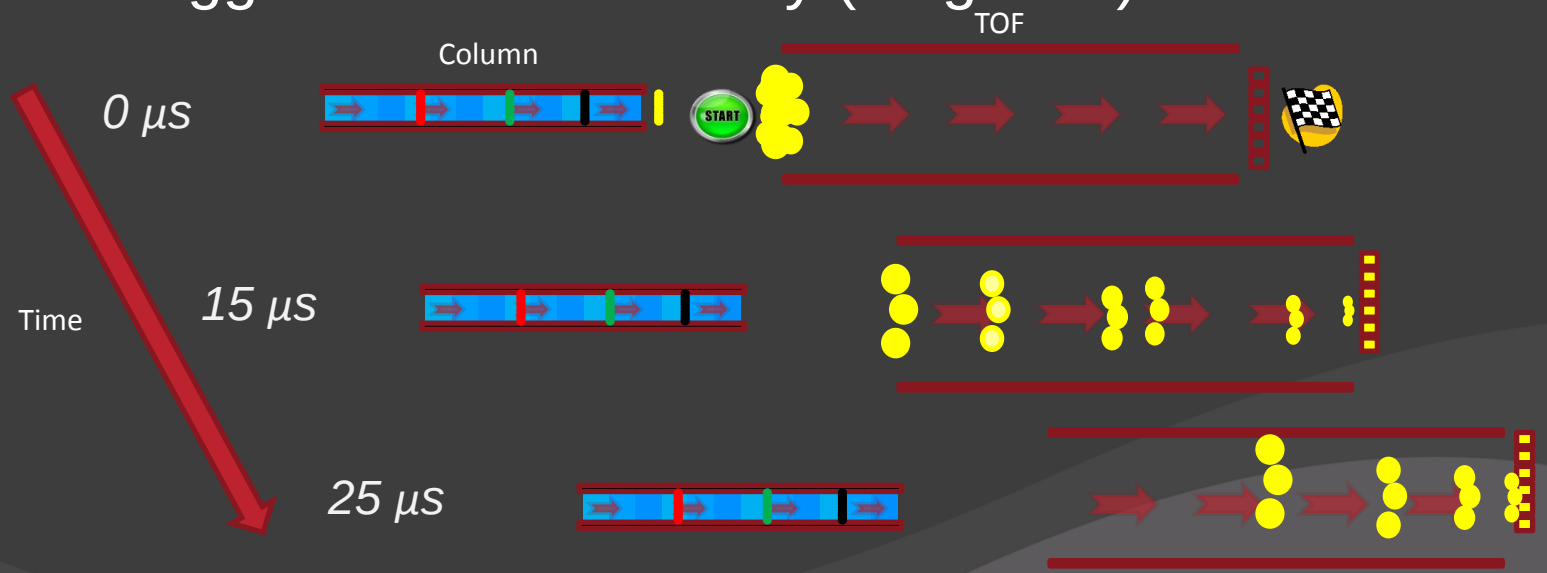
Key Points

Tandem mass spectrometry

- Unit resolution of ion precursor and product
  - e.g., an ion that weighs **234.1** can routinely be separated from an ion that weighs **235.1**
- Unique fragmentation of isobars can allow for differentiation
  - Oxymorphone vs. Morphine-N-oxide

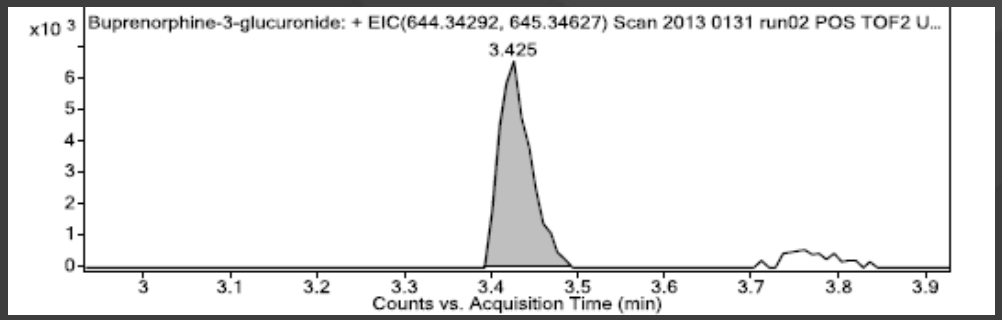
What is Time-of-Flight Mass Spectrometry?

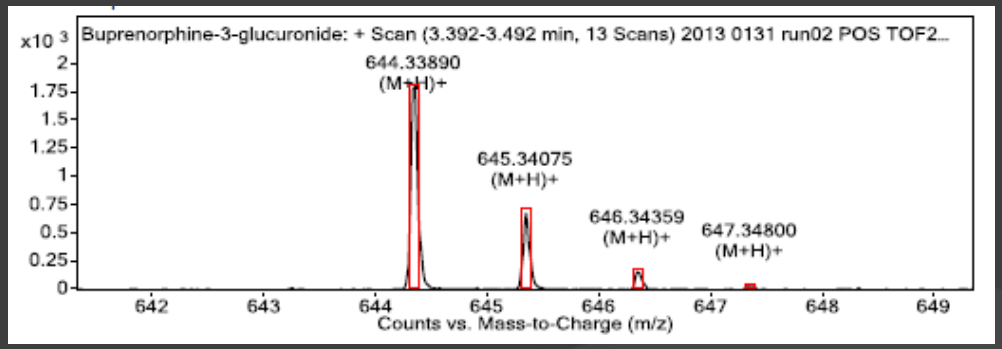
- Measures the mass of an ion by how long the drug takes to travel from start to finish
  - Smaller ions travel fast (short time)
  - Bigger ions travel slowly (long time)



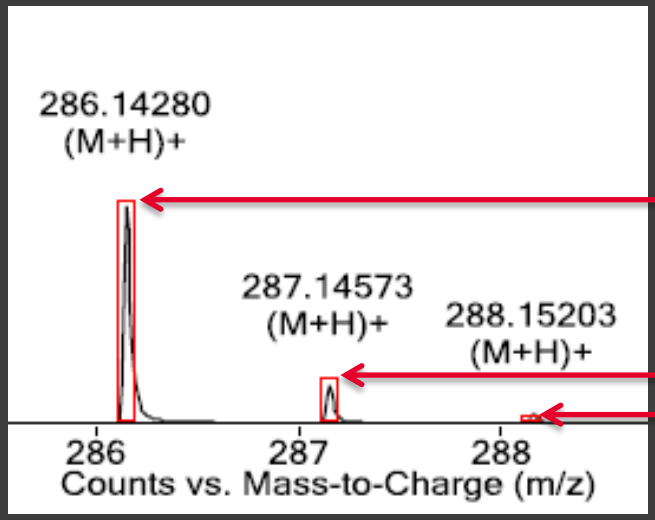
Acceptability Criteria for LC-TOF/MS Compound Identification

- Retention Time
- Mass error
- Abundance
- Score
  - Retention Time difference
  - Mass difference
  - Isotope spacing
  - Isotope abundance





Isotope abundance



Compound with only carbon 12 atoms

Compound with *one* carbon 13 atom

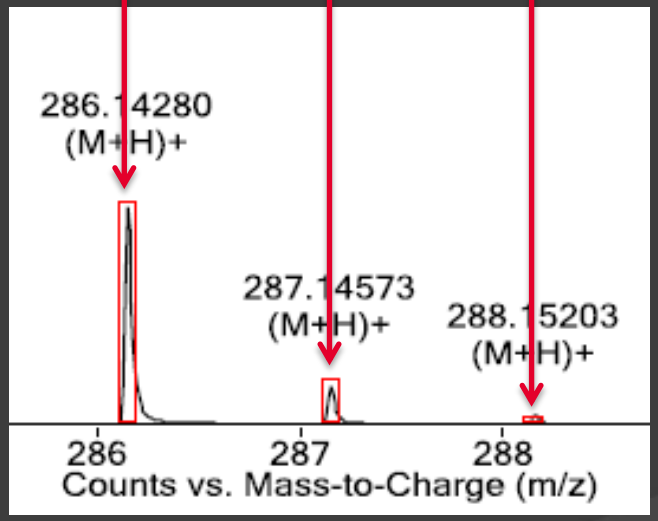
Compound with *two* carbon 13 atoms

Isotope spacing

Compound with only carbon 12 atoms

Compound with *one* carbon 13 atom

Compound with *two* carbon 13 atoms



TOF Limitations

- Isobars that are not chromatographically separated
  - Morphine-N-oxide & Oxymorphone
  - Endogenous interferences
- High Sensitivity vs. High Specificity
  - 5ppm error vs 75ppm error

One of Many Cool TOF Tricks

- Positive urine amphetamine screen that failed to confirm
- Known use of lebetalol
- Reprocessed data from the clinical run identifies a compound with mass of 328.1786

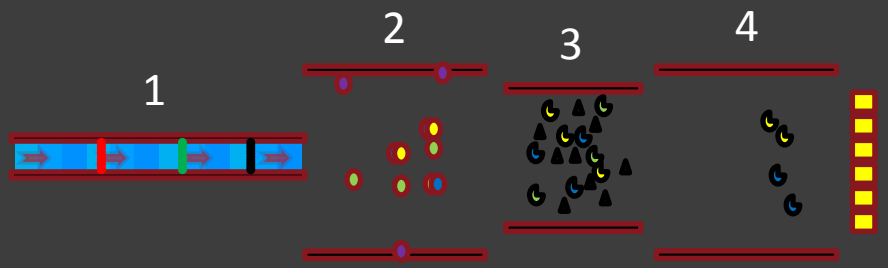
Key Points

Time-of-flight mass spectrometry

- Accurate mass of each drug is measured
  - e.g., a drug that weighs **234.1234** can routinely be separated from a drug that weighs **234.1175**
- Even natural carbon isotope distribution in the drugs can be detected and used for identification
  - e.g., ^{13}C instead of ^{12}C
- Detection limits are similar if not equal to tandem mass spectrometry

Tandem mass spectrometry vs. Time-of-flight mass spectrometry

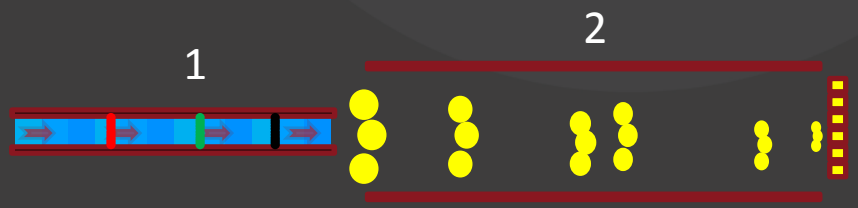
Tandem Mass Spec



1. Chromatographic separation
2. Filtered by mass
3. Collided with gas to form fragments
4. Fragments filtered by mass

Identification by fragmentation

Time-of-Flight



1. Chromatographic separation
2. Mass separation based upon time from start to finish

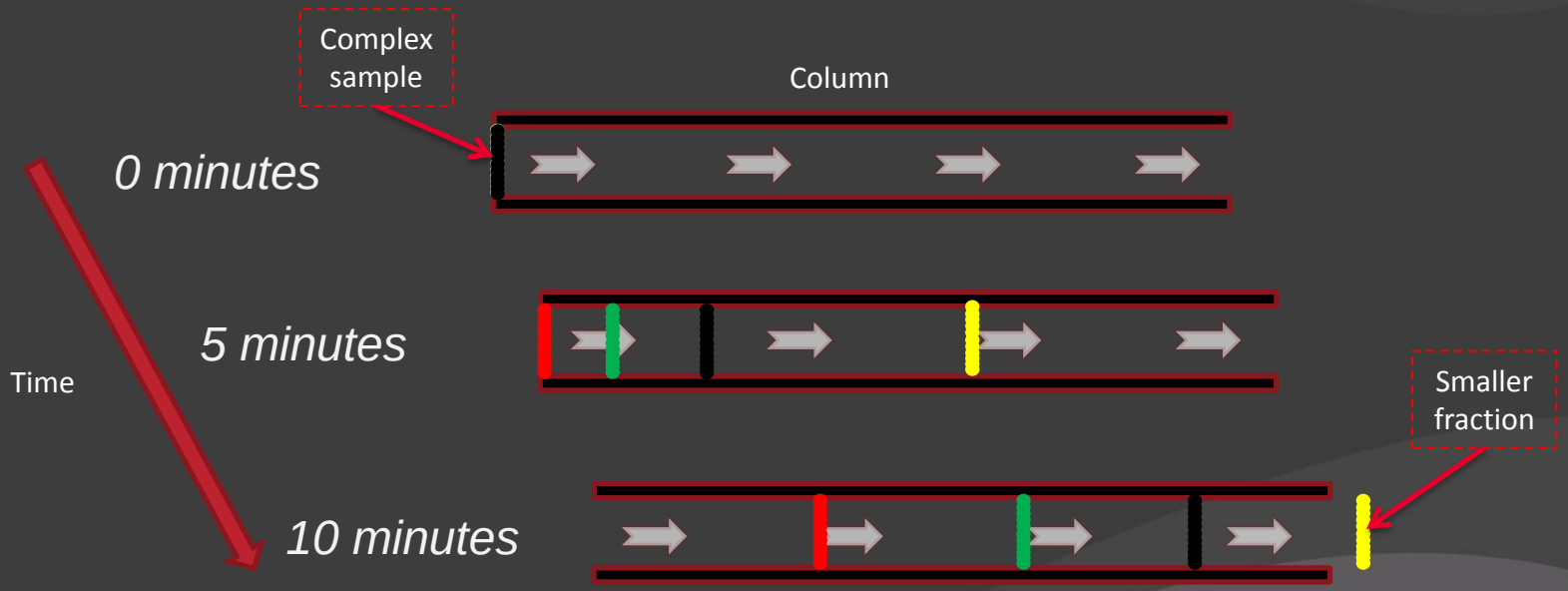
Identification by accurate mass and isotope profile

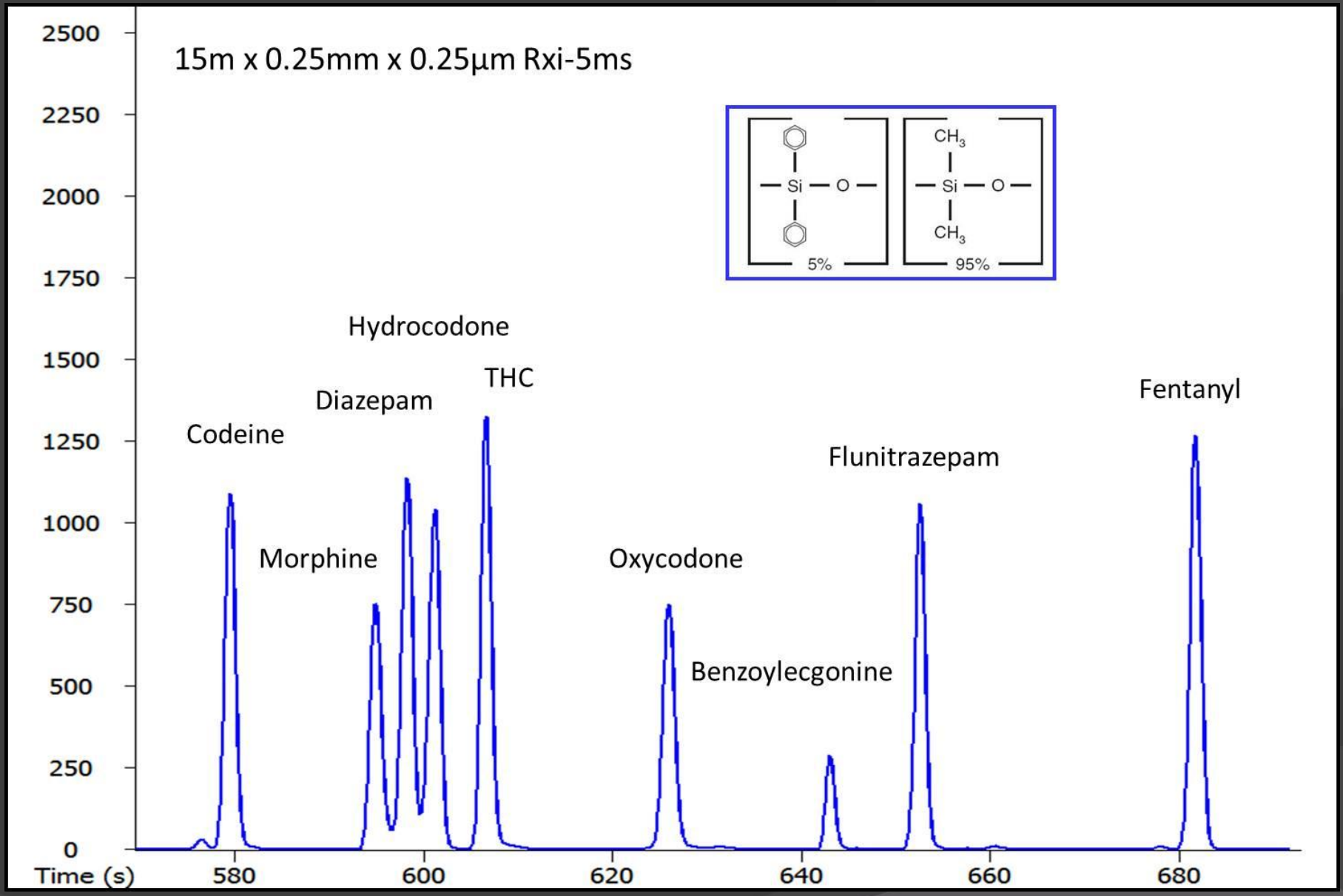
One Important Point

- Data is only as good as the separation
- Tandem Mass Spectrometry (MRM)
  - Inherent specificity can overcome less separation
  - Amobarbital & Pentobarbital
- TOF
  - Really struggles with specificity if separation is not adequate
  - No *easy* way to differentiate

Gas Chromatography

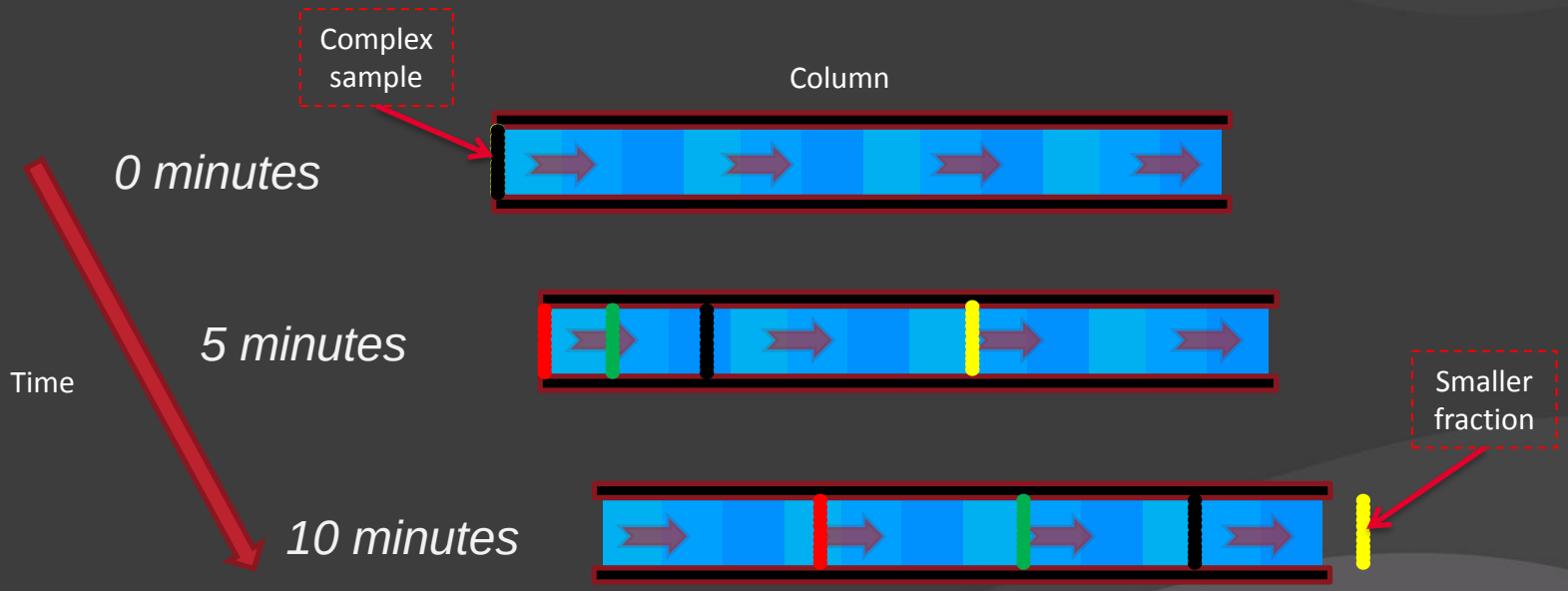
- Separation of a complex sample into smaller fractions before analysis

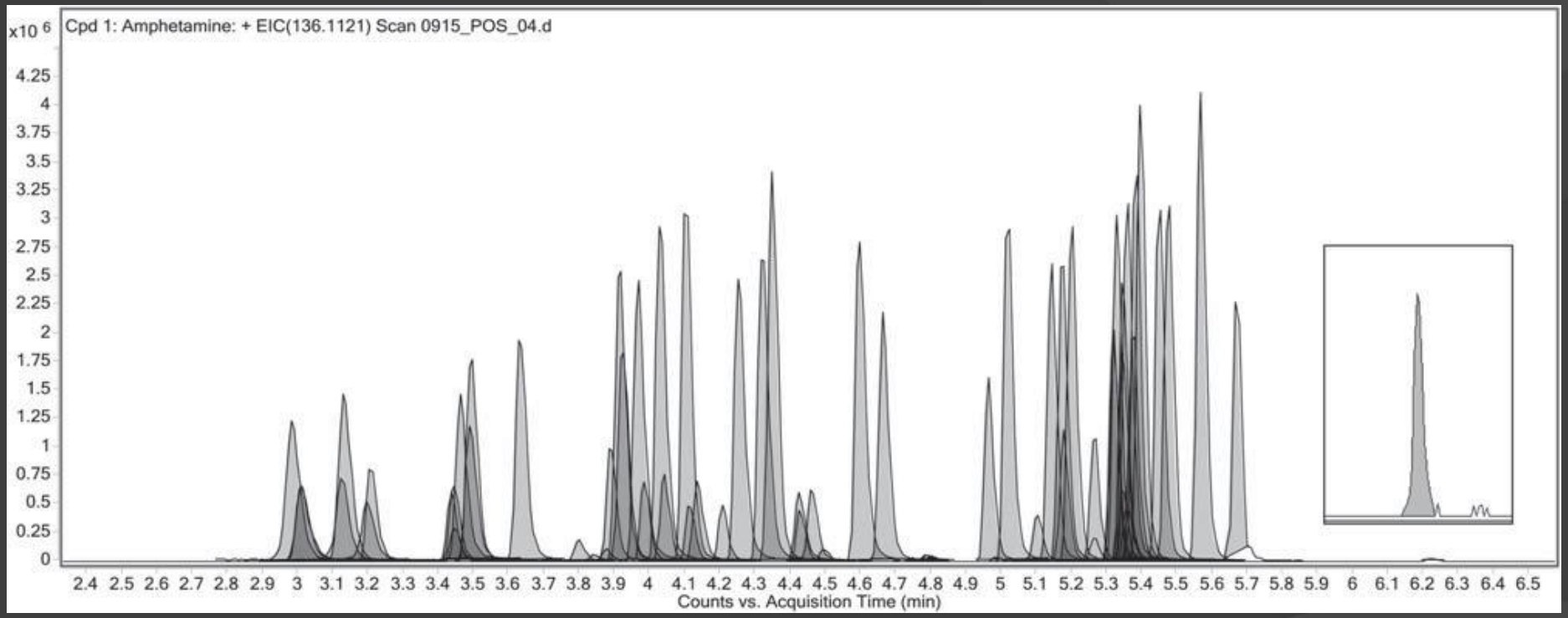



15m x 0.25mm x 0.25µm Rxi-5ms
Si
O
5%
CH3
Si
O
CH3
95%
Hydrocodone
THC
Diazepam
Codeine
Fentanyl
Flunitrazepam
Morphine
Oxycodone
Benzoylecgonine
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
Time (s)
580
600
620
640
660
680

Liquid Chromatography

- Separation of a complex sample into smaller fractions before analysis





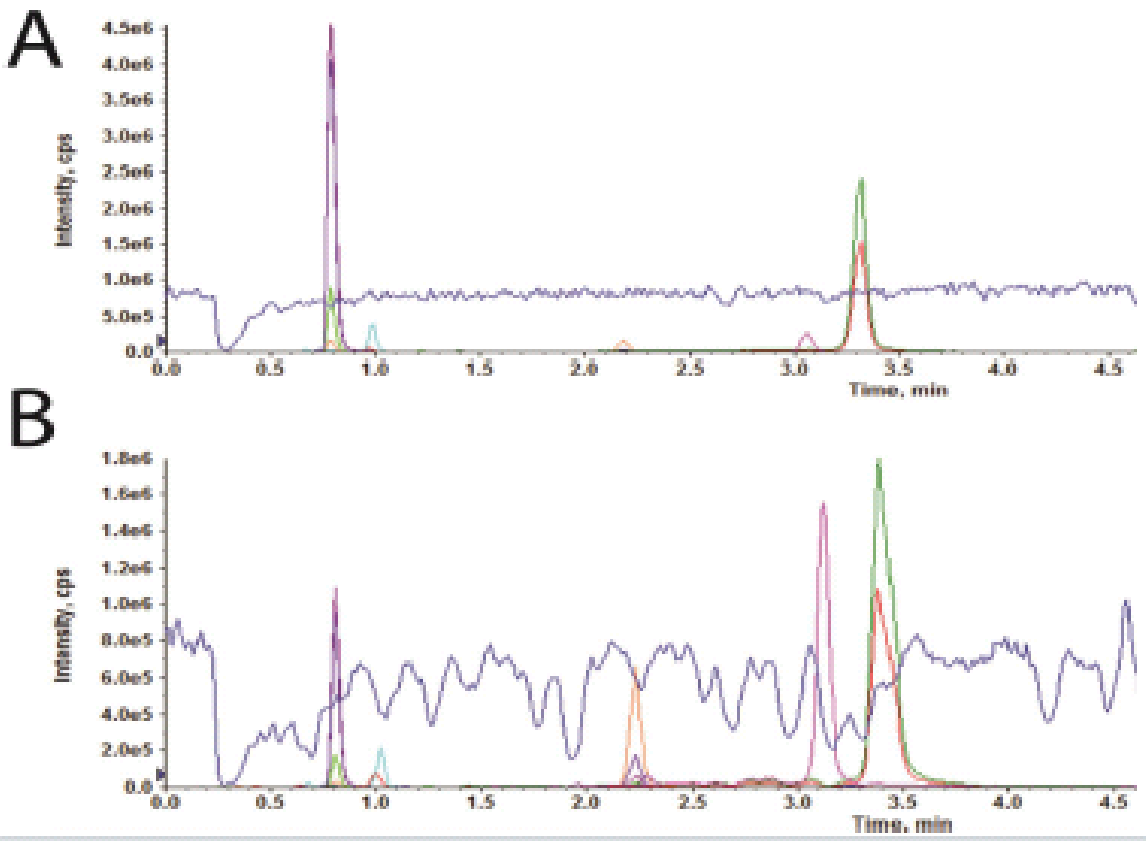
Marin, S. J., J. M. Hughes, B. G. Lawlor, C. J. Clark and G. A. McMillin (2012). Journal of Analytical Toxicology **36**(7): 477-486.

Nothing is perfect…

- Ion Suppression
- *Hotspot Carryover Contamination*

Ion Suppression

FIGURE 6. Unacceptable variation in matrix suppression profiles with dilute and shoot sample preparation. (A) A sample with minimal matrix effects and (B) a sample with considerable matrix effects.



Courtesy of Dr. Zlata Clark (MSACL 2014)

Internal Standards

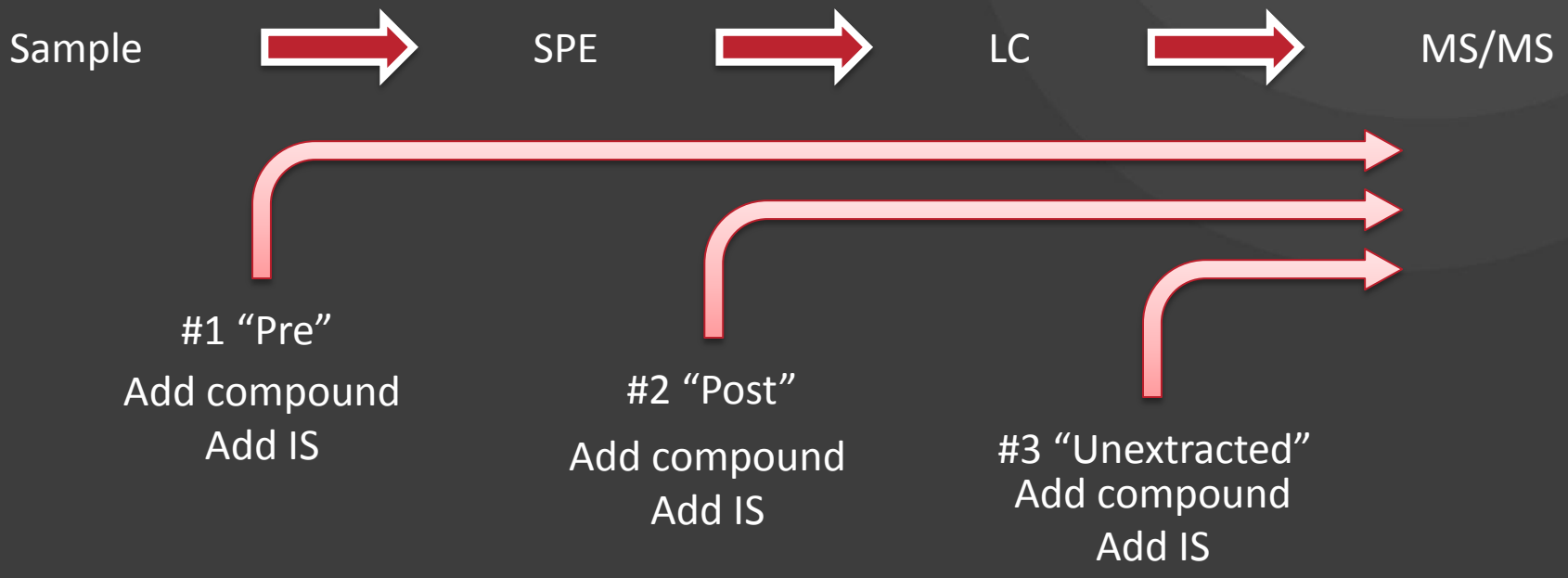
- Structurally related analogs
  - Deuterium
  - C13
- Act as a surrogate for the analyte of interest

- Analyte suppression = IS suppression

- Analyte / IS = normalized intensity

Analytical & Clinical Recovery

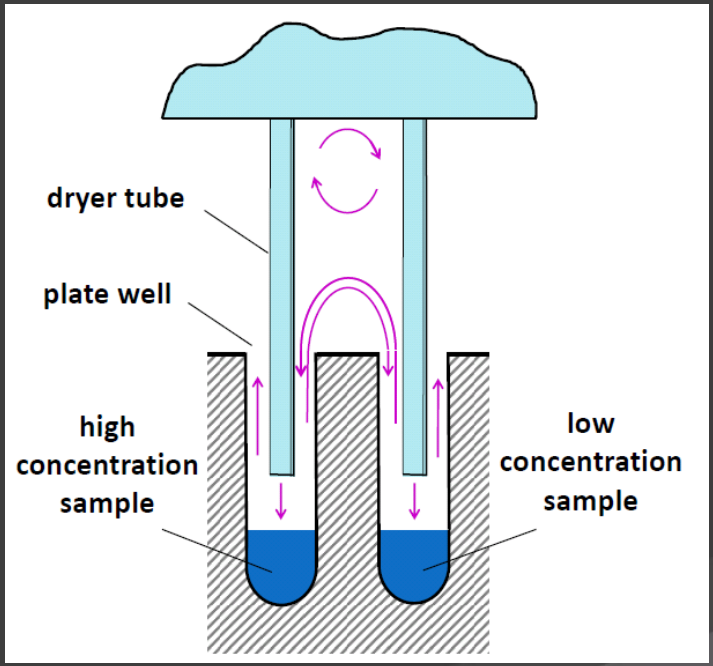
A Powerful Experiment



Result	Pre	%	Post	%	Unx	%
Area	250,000	50	350,000	70	500,000	100
Conc. (ng/mL)	249	99	248	99	250	100

Carryover from "Hotspots"

- If your method requires organic solvent evaporation by forced air you are likely having this problem.



Courtesy of Dr. Zlata Clark (MSACL 2014)

Clinical Impact of "Hotspots"

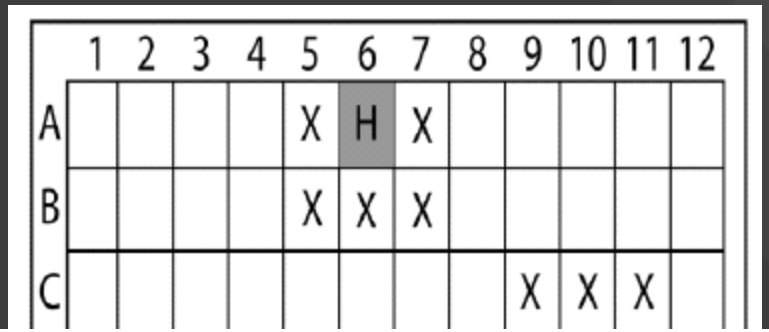


TABLE 1. HotSpot performance in over 76,000 clinical samples across multiple drug classes.

Analyte	Tested Samples	Re-extracted Samples	HotSpot True Positive	HotSpot False Positive	Positive Predicted Value (%)
Amphetamine	11,102	310	27	283	9
Methamphetamine		331	17	314	5
Codeine	57,020	23	12	11	52
Morphine		254	104	150	41
Hydrocodone		251	102	149	41
Hydromorphone		19	4	15	21
Oxycodone		513	241	272	47
Methadone	7497	7	1	6	14
EDDP		49	14	35	29
THC	1010	5	2	3	40
Total	76,629	1762	524	1238	30

Courtesy of Dr. Zlata Clark (MSACL 2014)

The Fundamental Question

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- What type of mass spectrometry is right for you?

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