

Clinical Mass Spectrometry Case Studies: How we can “Read Between the Lines”

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Disclosures

► None

Learning Objectives

- Recognize how mass spectrometry qualifier ions are used to confirm drug positive results.
- Identify atypical mass spectrometry results given a patient's clinical history and/or prescription patterns.
- Differentiate between patient compliance and patient non-compliance using mass spectrometry.

Questions to Ask

- Why is mass spectrometry a confirmation of immunoassay drug screens?
- What data can mass spectrometry provide that immunoassay drug screens cannot?

Case Studies Can Help Answer These Questions

- Case 1: Is it Methamphetamine?
 - False Positive Drug Screen Case
- Case 2: Where are my Benzos?
 - False Negative Drug Screen Case
- Case 3: Attempting to Keep Getting a Prescription Without Taking the Prescription
 - Diversion (Metabolite Case)

Drug Screens are Really Designed as a Rule-Out



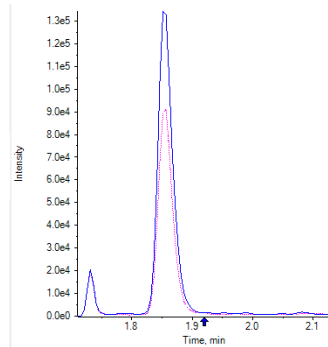
Case 1: Is it Methamphetamine? False Positive Drug Screen Case

- ▶ 28 y/o female
- ▶ Obese
 - ▶ BMI 35
- ▶ Rx for
 - ▶ Tylenol (Acetaminophen)
 - ▶ Adipex-P (Phentermine)
 - ▶ Oral Contraceptive (Levonorgestrel and Ethinyl Estradiol)
- ▶ Routine drug screen performed

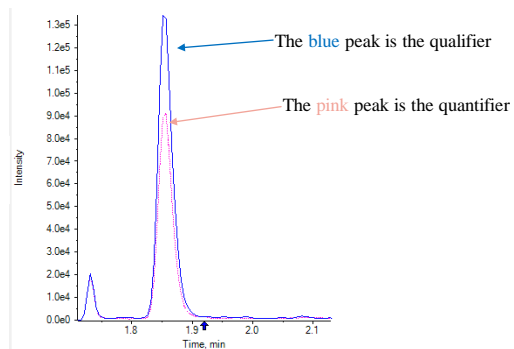
Is it Methamphetamine? False Positive Drug Screen Case

- ▶ Screen presumptively positive for Methamphetamine and negative for all other drugs
- ▶ Sent for confirmation by LC-MS/MS

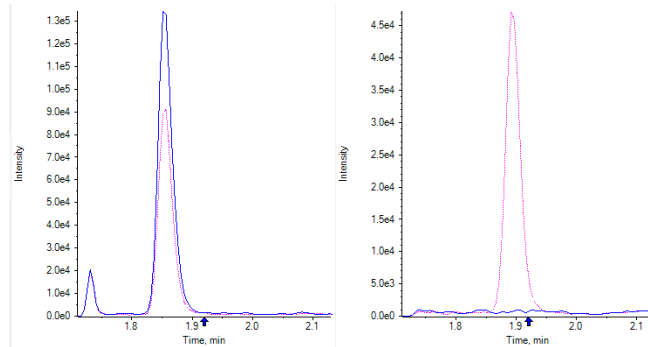
This is What a Typical Patient With Methamphetamine Looks Like



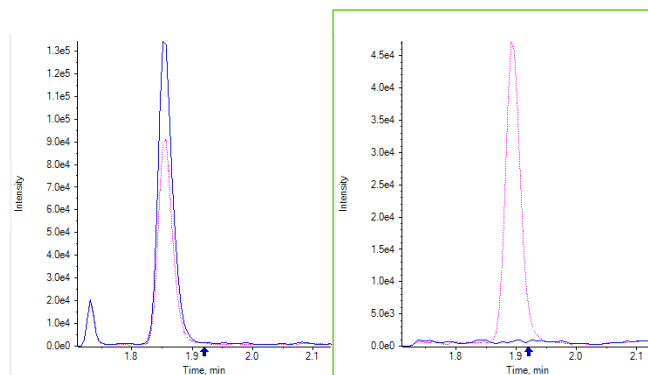
This is What a Typical Patient With Methamphetamine Looks Like



This is What our Patient's Sample Looked Like

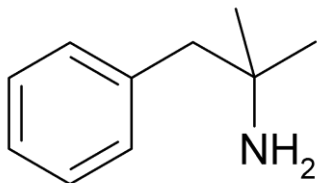


This is What our Patient's Sample Looked Like

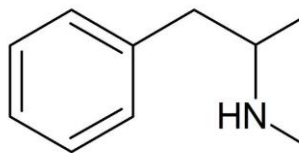


Negative for Methamphetamine and Amphetamine

Phentermine!



Phentermine



Methamphetamine

Patient's Original Screen was Confirmed a False Positive

- ▶ Patient was prescribed Phentermine.
- ▶ No Methamphetamine or Amphetamine was detected.
- ▶ Patient was maintained on medication regimen.

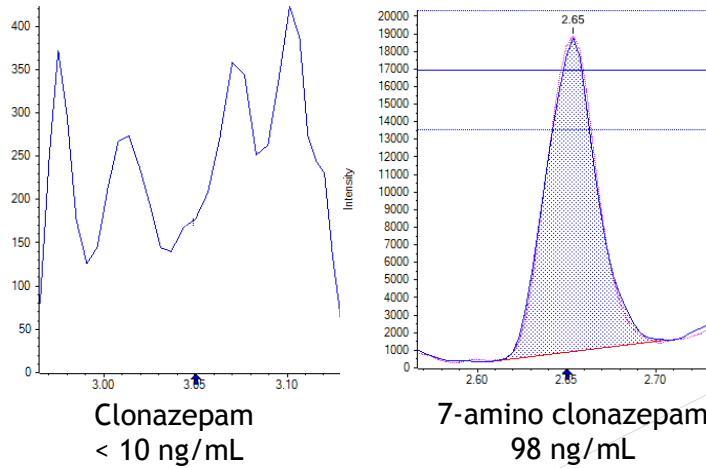
Case 2: Where are my Benzos? False Negative Drug Screen Case

- ▶ 66 y/o Male
- ▶ Hx anxiety and major depressive disorder
- ▶ Rx for
 - ▶ Klonopin (Clonazepam)
 - ▶ Lipitor (Atorvastatin)
 - ▶ Wellbutrin (Bupropion)
- ▶ Routine drug screen performed

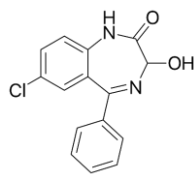
Where are my Benzos? False Negative Drug Screen Case

- ▶ Drug screen negative for all drugs
- ▶ Direct confirmation for benzos by LC-MS/MS ordered

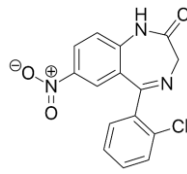
This is What the Patient's Sample Looked Like



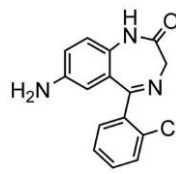
Why was the Screen Not Positive for Benzodiazepines?



Oxazepam



Clonazepam

7-amino
clonazepam

Patient's Original Screen was Confirmed a False Negative

- ▶ Patient was prescribed clonazepam.
- ▶ The metabolite of clonazepam, 7-amino clonazepam was detected.
- ▶ Screen cannot effectively detect the 7-amino clonazepam metabolite.
- ▶ Patient was maintained on medication regimen.

Case 3: Attempting to Keep Getting a Prescription Without Taking the Prescription Diversion (metabolite case)

- ▶ 58 y/o male
- ▶ Hx Hypertension, diabetes and chronic pain
- ▶ Rx
 - ▶ Zestril (Lisinopril)
 - ▶ Glucophage (Metformin)
 - ▶ Oxycontin (Oxycodone)
 - ▶ Lyrica (Pregabalin)
- ▶ Routine drug screen performed

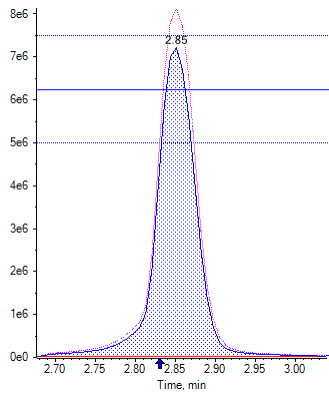
Attempting to Keep Getting a Prescription Without Taking the Prescription Diversion (metabolite case)

- ▶ Patient initially left clinic without leaving a urine sample
 - ▶ 9:15 a.m.
- ▶ Clinic refused to refill oxycodone and stated patient would be in violation of pain contract
 - ▶ Must show up before end of day to leave urine sample
- ▶ Agitated patient returned to leave sample
 - ▶ 10:45 a.m.

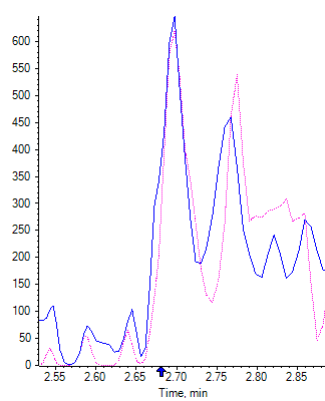
Attempting to Keep Getting a Prescription Without Taking the Prescription Diversion (metabolite case)

- ▶ Screen Presumptively Positive for oxycodone and opiates and negative for all other drugs
- ▶ Sent for Confirmation by LC-MS/MS

This is What the Patient's Sample Looked Like

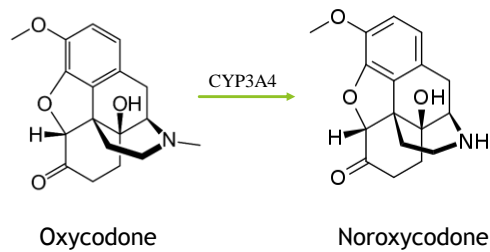


Oxycodone 51,850 ng/mL
after manual dilution



Noroxycodone <10 ng/mL

Metabolites do not lie



Roughly, can expect a 1:1 ratio in urine LC-MS/MS

Patient was Attempting to Hide Diversion

- ▶ Diversion
 - ▶ Not taking prescribed medication as often as indicated or never
 - ▶ Selling medication
 - ▶ Hoarding medication
 - ▶ Forgetting to take medication
- ▶ Avoiding urine drugs testing
- ▶ Adding medication directly to urine

Patient's Original Screen technically truly positive

- ▶ The screen picked up what it was supposed to
- ▶ But it can't tell if metabolites exist
- ▶ LC-MS/MS confirmed patient was not compliant with prescribed medication
- ▶ No future refills for oxycodone will be honored

Conclusions

- ▶ Drug screens are still an important part of clinical and forensic drug testing
- ▶ They do however, have limitations
- ▶ Mass Spectrometry based assays are necessary at confirmation of samples to be tested for drugs
 - ▶ Now able to identify atypical mass spectrometry results given a patient's clinical history and/or prescription patterns.
 - ▶ Now able to differentiate between patient compliance and patient non-compliance using mass spectrometry.

LC-MS/MS Implementation: Perspective From the Bench

Jeff Young, MLS(ASCP)^{CM}
Development Technologist
Providence Regional Laboratory-Oregon



Disclosures

- No Disclosures



Where we are Today

- **2 years and 5 months post installation of our first system**
 - 2 LC-MS/MS Systems in Production
 - 7 LC-MS/MS Assays with a Total of 40 Reportable Parameters
 - 49,741 Results Reported in 2017
 - 2.5 FTE dedicated to LC-MS/MS testing
 - Reduced Referral Spending by over \$700,000 Annually
- **2018 and Beyond**
 - Acquire and Validate a Liquid Handler
 - Develop enhanced reporting system for automating data review
 - Install 3rd LC-MS/MS system
 - Develop 3 new assays (10 reportable tests)



Overview

- Why LC-MS/MS? Justification?
- LC-MS/MS versus Immunoassay: A Quick Comparison.
- Where Did We Begin?
- How Did We Validate?
- How Did We Staff?



Why LC-MS/MS (Our lab's story)

- **2014/2015 Referred Testing Review**
 - Immunosuppressant Drugs (\$85K/year)
 - Common Urine Drug Confirmations (\$450-500K/year)
 - Serum Anticonvulsant Drugs (\$80K/year)
 - Methylmalonic Acid (\$150K/year)
 - Steroid Hormones (\$80K/year)
- **But it's not just \$\$\$.....**
 - Improve Service to Clinicians
 - Improve Result Turnaround Time

LC-MS versus Immunoassay

- **IA Benefits**
 - Faster
 - Random Access
 - Less Labor
 - FDA-Cleared
- **IA Drawbacks**
 - Less Specific/Risk of False Positives
 - Less Sensitive
 - Costly Reagents
- **LC-MS/MS Benefits**
 - Gold Standard
 - Definitive
 - Lower Reagent Costs
 - Broad Menu Options
- **LC-MS/MS Drawbacks**
 - Manually Intensive
 - Slower/Batch Driven
 - LDT Validation
 - Hardware Cost

How did we get started?

- **Start Small**-Evaluate Clinical Need versus \$\$\$
- **Began with Tacrolimus**
 - Easy Sample Extraction
 - Fast Run-Time
 - Pre-existing FDA-Cleared Kit
- **Moved to Urine Amphetamines Confirmations**
 - Improved Outcomes and Discharge TAT for Maternity
 - Dilute and Shoot Extraction
 - Utilized Same Mobile Phases
 - Expanded Menu while Preserving Development Time for Analyzer



Assay Validation Overview

- **FDA-Cleared**
 - Linearity
 - Precision
 - Correlation/Accuracy
 - Reference Range
 - Carryover
- **Lab Developed Test (LDT)**
 - Literature Review
 - Method Development/Tuning
 - Linearity
 - Precision
 - Correlation/Accuracy
 - Reference Range
 - Carryover
 - LOB, LOD, LOQ
 - Stability (Cal, QC, Sample, Extract, SST)
 - Freeze/Thaw
 - Matrix Effect/Ion Suppression
 - Interference Testing



Embrace Failure

- **Validation Success CAN Lead to Failure**
 - Methylmalonic Acid
 - We became overconfident.
 - Validation of a rapid protein crash extraction appeared to be great.
 - Post validation column change caused chromatography degradation.
 - Revalidated using a more complicated derivatization extraction.....

ON MULTIPLE COLUMNS



Testing Staff

- **LC-MS/MS is Different from Routine Automated Testing**
 - Not for everyone. Gauge Interest and Long Term Ability and Compatibility.
 - Keep the area specialized. We do not cross train.
 - Consider both SME's and New Graduates.
 - Consider Laboratory Assistant for Sample Prep.
- **Maintain Flexibility and Consider Crisis Management**
 - **1 Lead / 1.5 Toxicologists:** Production and New Development.
 - **Development Tech:** Maintains Competency: Covers PTO Days and Inventory
 - **Director:** Maintains Competency, Performs Data Review and Validation Studies



Summary

- **Implementation of LC-MS/MS is Achievable!**
 - Understand the Benefits and Drawbacks.
 - Start Small.
 - Evaluate Dollars and Clinical Needs.
 - Expect Extended Validation Periods.
 - Hire for Long-Term Growth.
 - Learn from Failure and Celebrate Success.



Questions or Discussion

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A BRIEF INTRODUCTION TO THE BASICS OF LC-MS/MS IN CLINICAL LABORATORY

NANDU CHINDARKAR, PhD, DABCC
KAISER PERMANENTE REGIONAL LABORATORY
BERKELEY, CA

DISCLOSURES

None

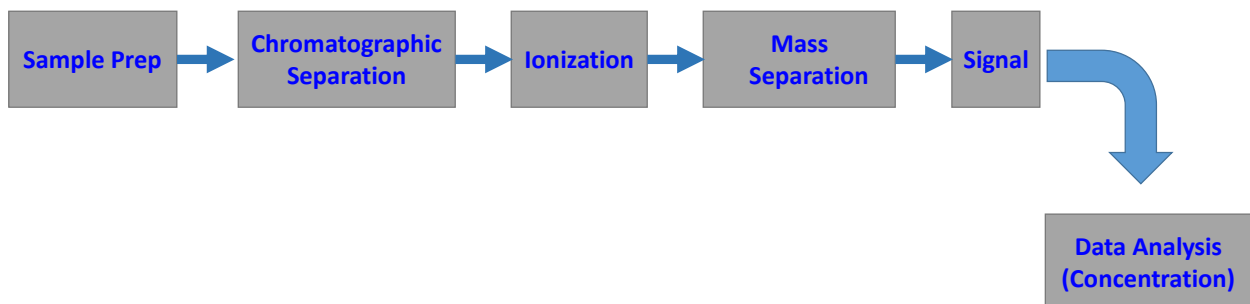
ABBREVIATIONS

- **LC** - Liquid Chromatography
- **ESI** - Electrospray Ionization
- **MS** - Mass Spectrometry
- **CID** - Collision-Induced Dissociation
- **MRM** - Multiple Reaction Monitoring
- **IS** - Internal Standard
- **m/z** - mass-to-charge (z) ratio

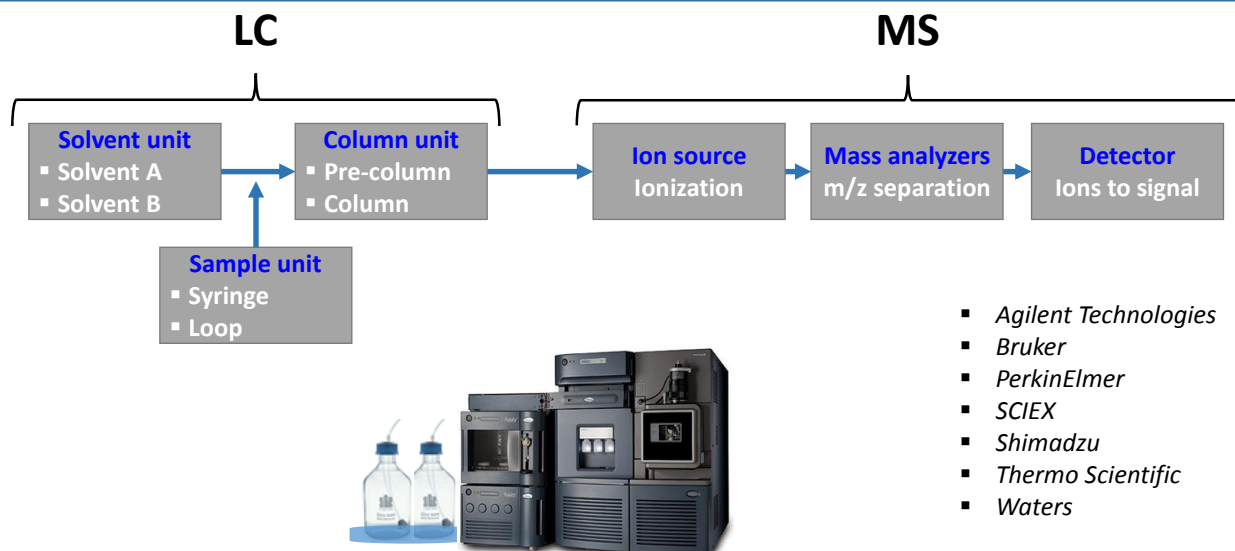
QUANTITATIVE LC-MS/MS : MAJOR ANALYTES

- **Drugs of abuse**
 - Amphetamines, Opiates, Benzodiazepines, etc.
- **Therapeutic drug monitoring (TDM)**
 - Tacrolimus, Vancomycin, Digoxin, etc.
- **Hormones**
 - Vitamin D, Testosterone, Estrogen, Cortisol etc.
- **Other small molecules**
 - Methylmalonic acid, Catecholamines, Metanephrines, etc.

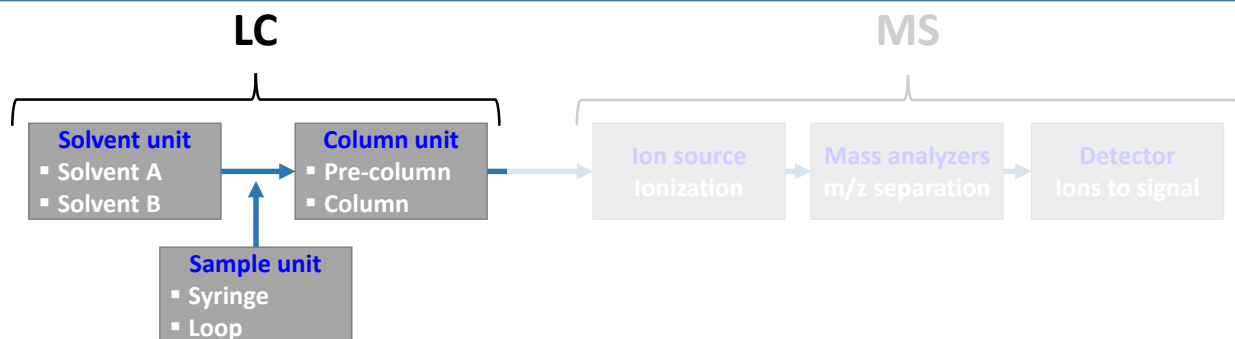
QUANTITATIVE LC-MS/MS: WORKFLOW



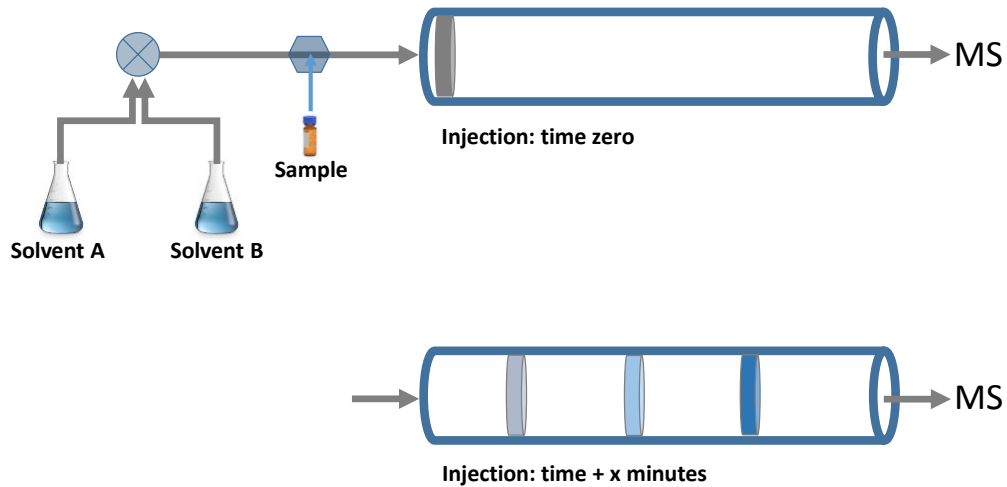
COMPONENTS OF LC-MS/MS



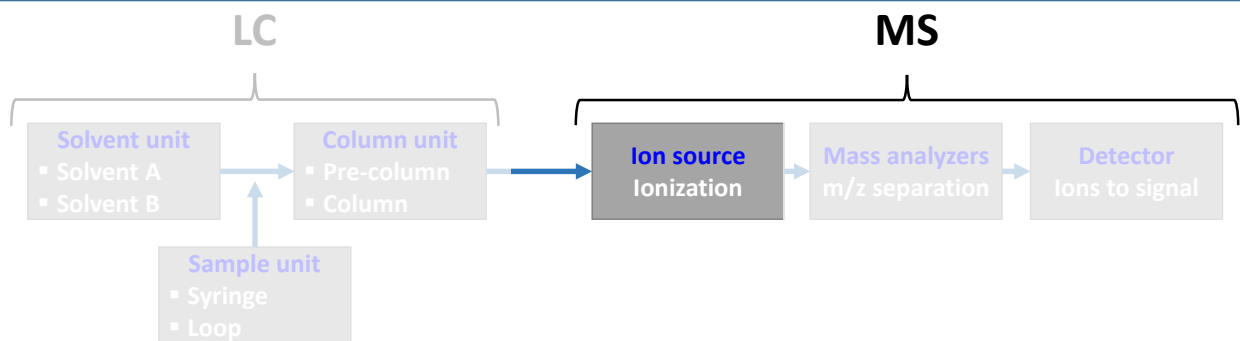
COMPONENTS OF LC-MS/MS



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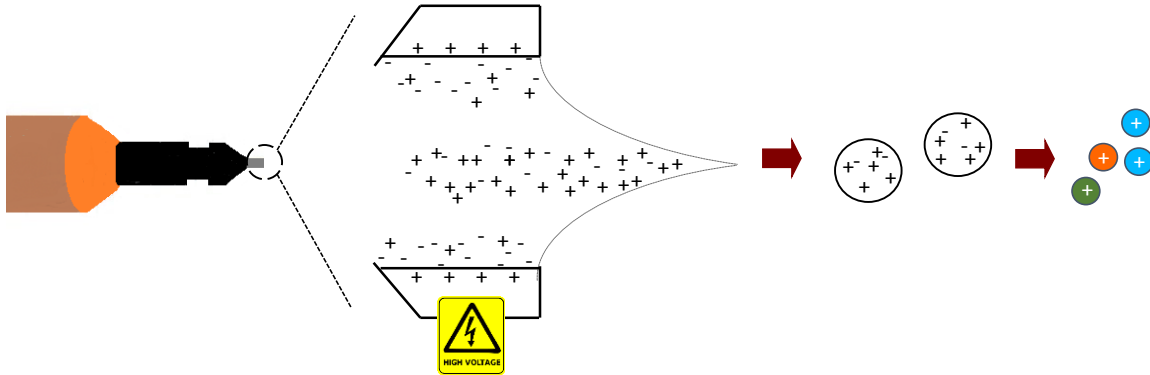


COMPONENTS OF LC-MS/MS

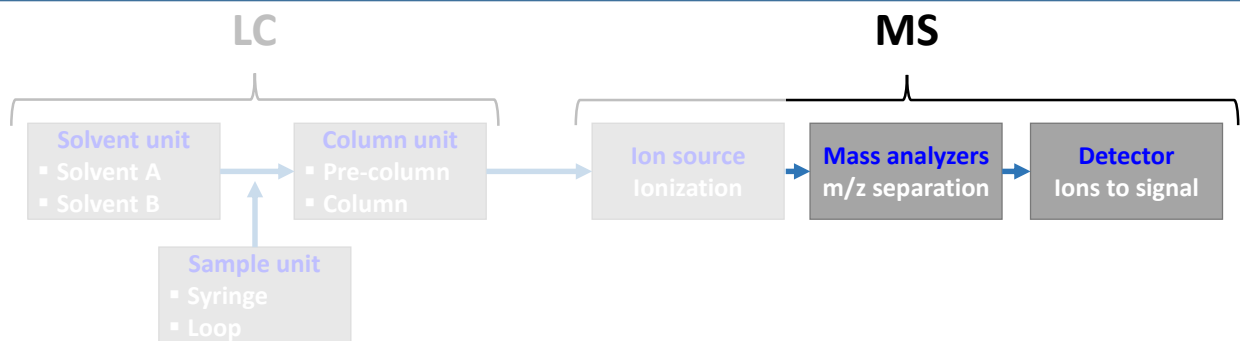


COMPONENTS OF LC-MS/MS

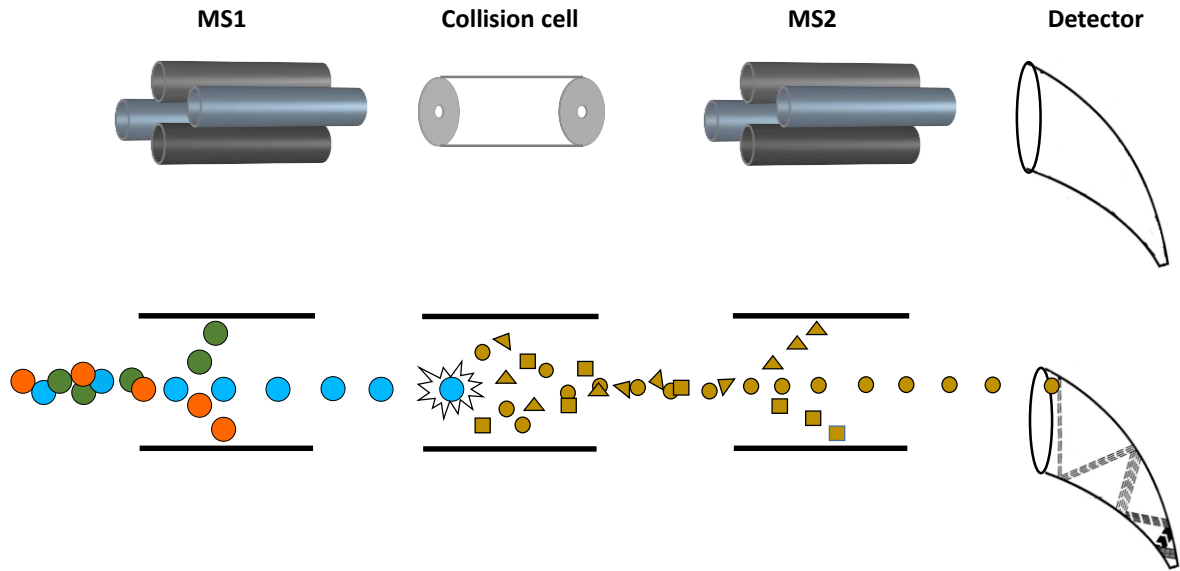
Electrospray Ionization (ESI)



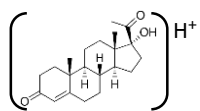
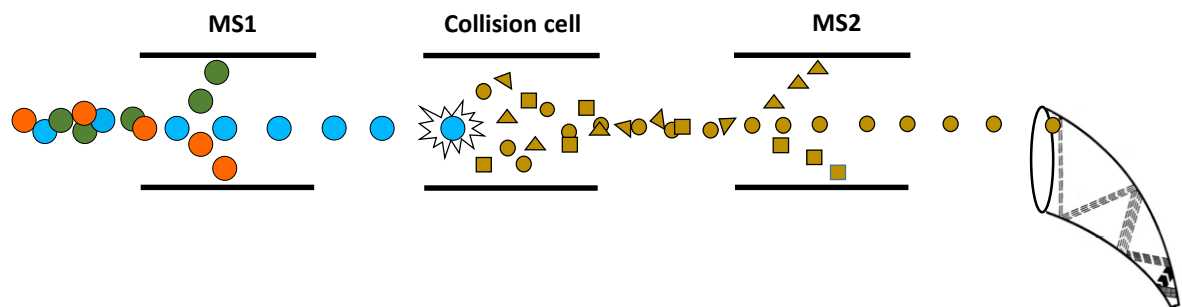
COMPONENTS OF LC-MS/MS



COMPONENTS OF LC-MS/MS



COMPONENTS OF LC-MS/MS : SELECTIVITY



m/z 331

331

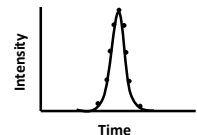
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331 → 295

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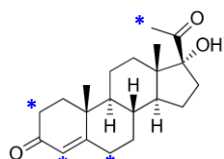
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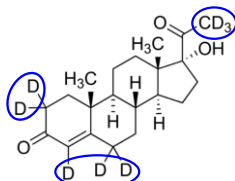
LC-MS/MS: INTERNAL STANDARD (IS) & QUANTITATION

- A chemical substance that is added in a constant amount to calibration standards, controls, and unknown samples
- Corrects for the loss of analyte during sample preparation (e.g. SPE) or analysis (matrix effect)
- Physicochemical properties similar to the analyte (yet differentiable by MS)



17α-Hydroxyprogesterone
MW = 330.5 g/mol

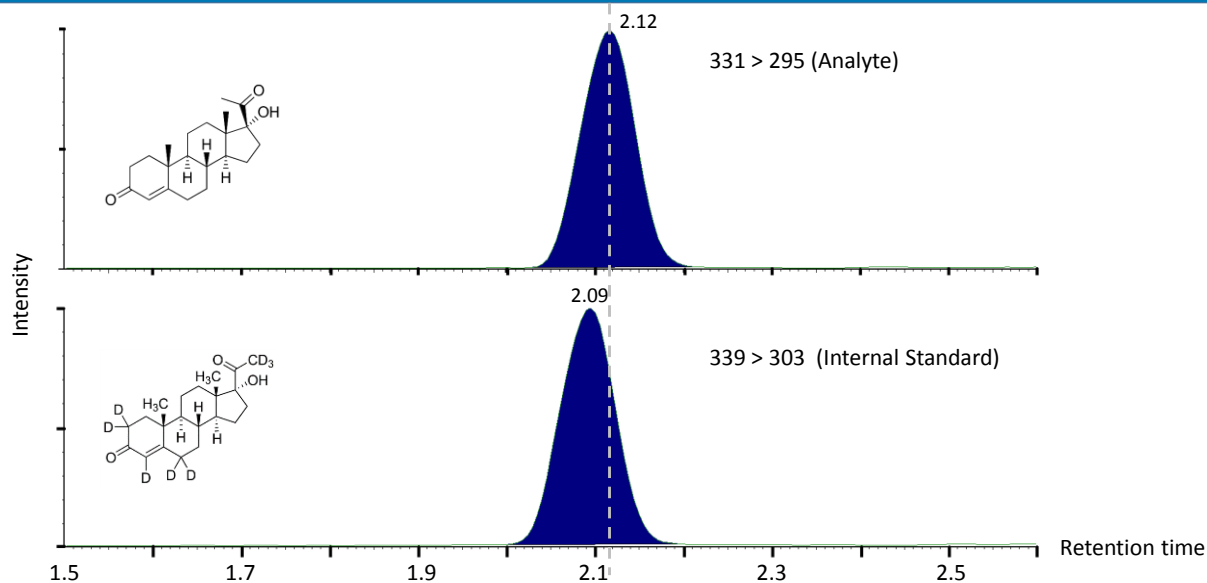
331 → 295



17α-Hydroxyprogesterone-D8
MW = 338.5 g/mol

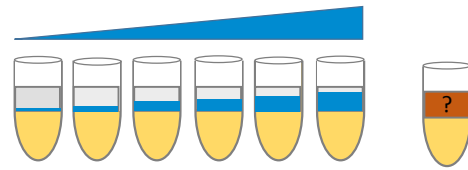
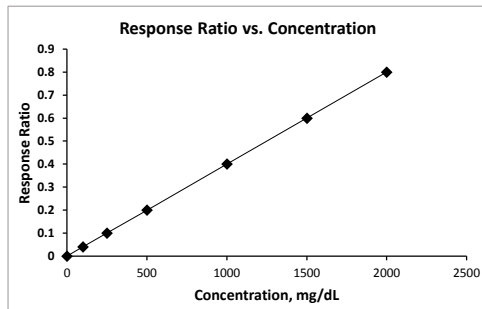
339 → 303

LC-MS/MS: INTERNAL STANDARD (IS) & QUANTITATION



LC-MS/MS: INTERNAL STANDARD (IS) & QUANTITATION

- Response ratio: analyte peak area/IS peak area
- Calibration plot – response ratio vs. assigned concentration of standards



THANK YOU!



Speaker and Presentation Evaluations for
Saitman/ Young/ Chindarkar using Survey
Monkey

<https://www.surveymonkey.com/r/LKTLQQC>

Please let us know what training resources you need

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