Sample Preparation – A Practical Approach for Robust Analyses

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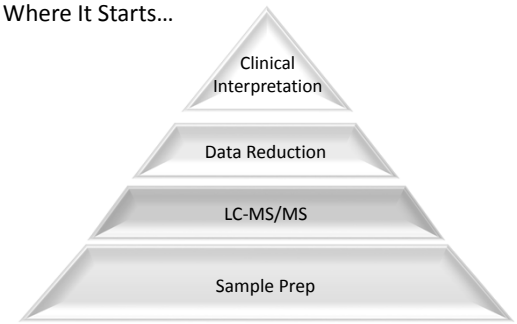
Learning Objectives

- Leverage strengths and weaknesses of common sample preparation techniques used for clinical liquid chromatography tandem mass spectrometry, LC-MS/MS
- Mitigate effects of sample matrix
- Implement sample prep techniques for different sample types, e.g., plasma, urine, oral fluid, meconium
- Apply methodologies compatible with the dynamic range of analytes in complex matrices

Sample Prep for Clinical MS: Where It Starts…



Effects of Sample Matrices

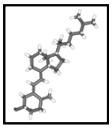
- Interference – is it the right peak?
- Suppression – can the cutoff be met?
- Variability among patient specimens
  - Different creatinine level, urea, total protein
- Deteriorates instrument performance
  - Mass spectrometers can measure any ionized analyte
- Causes the injector and mass spectrometer to get dirty

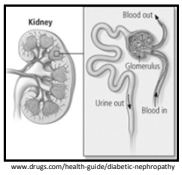
Sample Prep for MS: Matrices

Toxicology
- Urine
- Oral Fluid
- Plasma, Serum, Whole Blood
- Meconium, Cord Tissue

Endrocrinology
- Plasma
- Serum

Proteomics
- Plasma
- Serum
- Tissue



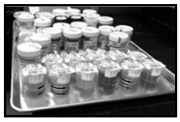


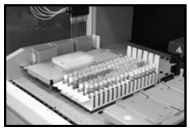
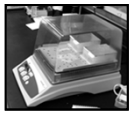
www.drugs.com/health-guide/diabetic-nephropathy

Sample Preparation Sequence and Evaluation

- Add internal standard early in process (prior to extraction)
- Determine whether interferences and suppression are adequately removed
- Include variety of patient specimens
- Investigate specimens with low recovery or excessive noise
- Perform large enough study to address problem points
- Ensure test has adequate precision over time

General Toxicology Sample Preparation Process



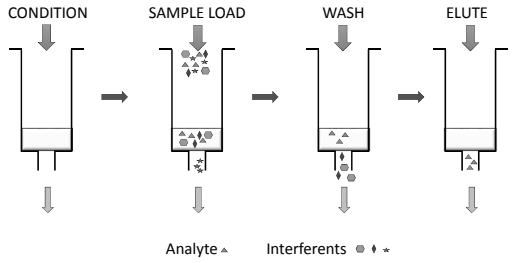



Sample Cleanup
Dry-Down
Reconstitution

LC-MS/MS

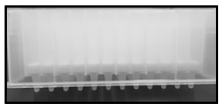
Sample Cleanup

› SPE

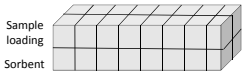


Sample Prep

› Preparation Method Advantages



- Solid Phase Extraction: SPE
  - › Concentrate sample
  - › Cleans samples to support column longevity
  - › Lower limits of detection
  - › Decreased interferences
  - › Reduced ion suppression
- Chemistry: cation or anion exchange
  - › Removes salts
- Filtration: size exclusion
- Steps: condition plate, load samples, wash samples, elute (capture in clean well plate)



Sample Prep

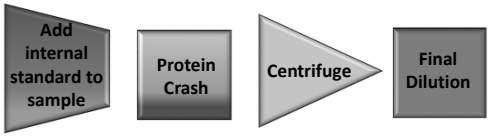
› Preparation Method Advantages

- Supported Liquid Extraction: SLE
  - › Solvent exchange (aqueous to organic)
  - › Straight forward
  - › Decreased prep time
  - › Concentrate sample
  - › Mitigates matrix effects
  - › Cleans samples to support column longevity
- Chemistry
  - › Mechanism of sample cleanup
- Filtration
  - › Removal of large molecular weight components

Sample Prep

› Preparation Method Advantages

- Dilute and Shoot
  - › Offers fast sample prep with minimal consumables
  - › Often includes a protein crash step prior to centrifugation
  - › Dilution of prepared sample minimizes the presence of salts and matrix effects
  - › Compatibility is determined by LOD requirements and instrument performance

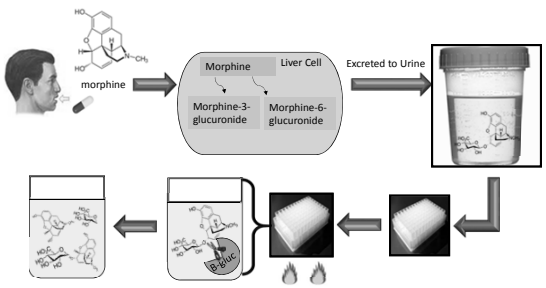


Sample Prep

› SPE
› SLE
› Dilute and Shoot

	Dilute and Shoot	SLE	SPE
Prep Time	↓	↔	↑
Process Complexity	↓	↔	↑
Consumables	↓	↑	↑
Sensitivity	↓	↔	↑
Column Life	↓	↔	↑
Potential Matrix Interferences	↑	↔	↓

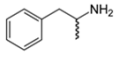
Sample Prep: Hydrolysis



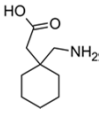
Application: Dynamic Range of Analytes in Urine

Typical Limits of Quantitation

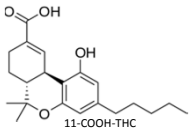
- Benzodiazepines
  - 50-100 ng/mL
- Opiates/Opioids
  - 50-100 ng/mL



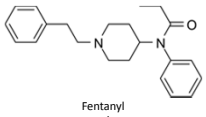
Amphetamine
100 ng/mL



Gabapentin
500 - 1000 ng/mL



11-COOH-THC
20 ng/mL



Fentanyl
4 ng/mL

Table of SAMHSA Cutoff Levels

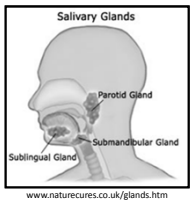
Analyte	Oral Fluid Cutoff (ng/mL)	Urine Cutoff (ng/mL)
6-acetylmorphine	2	10
codeine/morphine	15	2000
cocaine/benzoylecgonine	8	100
delta-9-tetrahydrocannabinol (THC)	2	15*
phencyclidine	2	25
Amphetamine/methamphetamine	15	250
Methylenedioxymethamphetamine (MDMA)	15	250
Methylenedioxyamphetamine (MDA)	15	250
Methylenedioxyethylamphetamine (MDEA)	15	250
oxycodone/oxymorphone	15	50
hydrocodone/hydromorphone	15	100
*Delta-9-Tetrahydrocannabinol-9-carboxylic acid (THCA)		

References:
http://www.gpo.gov/fdsys/pkg/FR-2015-05-15/pdf/2015-11523.pdf
http://www.gpo.gov/fdsys/pkg/FR-2015-05-15/pdf/2015-11524.pdf

Sample Prep: Toxicology

› Oral Fluid

- Proposed Substance Abuse and Mental Health Administration SAMHSA approved matrix
- Neat vs. buffered sample extraction
- Hydrolysis not necessary
- Lower limit of detection required
  - › Detector must be able to perform with adequate signal/noise and signal intensity in the low concentration range
  - › Sample preparation must have an efficient recovery, e.g., not irreversibly bind with the analyte



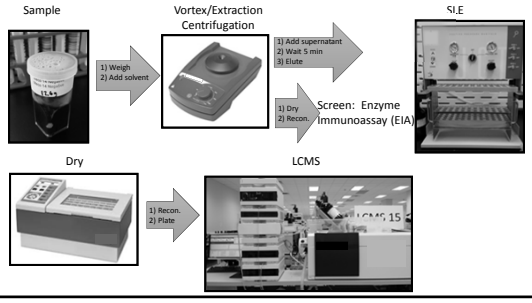
www.naturecures.co.uk/glands.htm

Sample Prep: Toxicology

› Serum, Plasma, Whole Blood Advantages

- Enables therapeutic drug monitoring
- Protein Crash
  - › Limits of detection
- Dilute and Shoot
  - › Speed
  - › Range of analytes
  - › Varying matrices

Tailored Implementation: Meconium



Sample Prep: Endocrinology

› Serum/Plasma

› Antibody-Mediated Detection Methods
- Cross-platform variability, antireagent antibodies, the hook effect, and insufficient specificity

› Optimization has reduced the characteristic disadvantages

J. Immunol. Methods. **2009**, 347, 3-11.
Am. J. Clin. Path. **2011**, 136, 609-616.

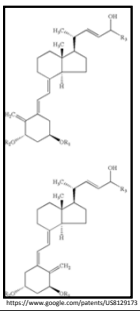
Sample Prep: Endocrinology

› Hypovitaminosis D

› Vitamin D_2 versus Vitamin D_3
- LC-MS/MS methods: differentiate epimers

› Sample Prep Application:
- Vortex
- Extract with *n*-heptane
- Centrifuge (4 m, 3,000 rpm)
- Organic layered removed, evaporate (N_2)
- Reconstitute in EtOH
- Vortex

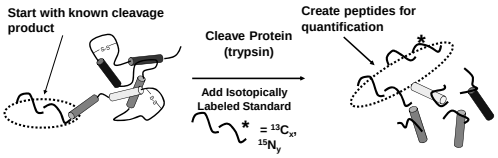
› Rapid, sensitive LC-MS/MS method for measuring serum levels of 25(OH)D_2 and 25(OH)D_3 at nanomolar concentrations with novel ISTD



https://www.google.com/patents/US8129173

Am. J. Clin. Path. **2006**, 125, 914-920.

Sample Prep: Bottom Up Proteomics



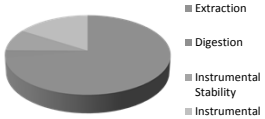
1. Aliquot specimen(s)
2. Denature protein with urea (6 Molar)
3. Adjust pH and reduce with 10 mM dithiothreitol
4. Alkylate with 30 mM iodoacetamide
5. Digest protein with trypsin or LysC
6. Acidify samples and centrifuge 14000 x *g*
7. Transfer to microplate or vials for LC-MS/MS analysis

J. Proteome Res., **2004**, 3, 644-652.

Sample Prep: Proteomics

- Tissue Homogenization
  - Tissue placed in a 96-deepwell plate
  - Homogenization buffer added
    - 8M Urea, 10 mM DTT in 50 mM Tris-HCl
  - Homogenized
    - Retsch Mixer Mill MM 400 (20 Hz, 2 m)
  - Aliquots filtered
    - 1.2 um low-protein binding filter plates
- Largest contribution to variability came from the extraction component:
  - Sample dissection
  - Homogenization

Contributions to Variability in Proteomics Pipeline



J. Proteome. Res. **2013**, 12, 2128-2137.

Conclusion

- Sample preparation is an essential component of clinical mass spectrometry
- Sample matrix effects are mitigated by the appropriate sample preparation technique
- Analyte specific cutoff levels should be considered when choosing the sample preparation method
- The performance of the separation system and mass spectrometry are essential considerations
- Cost of sample preparation must be balanced with maintenance costs of analytical instrumentation

Acknowledgements and Contact

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