Clinical Diagnostics: Innovation, Validation, Implementation, and Operation by Mass Spectrometry

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Course Outline and Structure

Style: Workshop/dialog oriented
Detailed and example driven
Reinforcement of content for each session

Day 1: Step 1 LC-MS/MS: the experiment and terminology
Step 2 Interfaces, Infusion, mobile phases and LC
Step 3 Extraction and Selectivity
Step 4 Gotcha’s and Throughput
Step 5 Q&A – Your problems discussed

Day 2: Step 1 Validation guidance and pre-val stress testing
Step 2 Selectivity and Interferences
Step 3 Accuracy, Precision and Linearity
Step 4 Ruggedness, Stability, Transfer and Launch
Step 5 Q&A – Your problems discussed
Real-World Examples and Troubleshooting

Double Blank Contaminated

*Gotcha – Contamination in double blank in both Transitions for Norepinephrine*

Experiments –
- Cleaned all containers
- Fresh Solvents in preparation
- Pre-washed all contact materials
- Evaluated lot-lot variance in materials
- Autosampler (AS) carry-over? AS wash solvents contaminated?
- Is it really Norepinephrine even with transition ratios that match standards?

Answer: NO

How do we know: We changed LC separation (stationary phase), BUT sensitivity was not good enough

Solution – Peak width of 1.2 seconds (unpredicted by Van deemer), changed pore size ONLY

Multiplexed Steroid Analysis

*Testosterone Serum (1) LLE-TFC-LC-MS/MS*

*Cortisol Serum (2) TFC-LC-MS/MS*

*Cortisol Urine (3) LLE-TFC-LC-MS/MS*

*Progesterone & 17OHProgestosterone Serum (4) LLE-MS/MS*

Quant/Qual – 60+ Amino Acids 1 prep 3xLC

RF#1

HILIC

RF#2

Quantitative

Qualitative

Intra-Assay Inaccuracy

Intra-Assay Imprecision

Intra-Assay Inaccuracy

Intra-Assay Imprecision

Intra-Assay Inaccuracy

Intra-Assay Imprecision

Accuracy – Comparison to Gold Standard Method

FDA approved method: IEX SPE, Ion Pairing LC-ECD, 20 min inj/inj

Gotcha – Discordant results observed in inter-assay correlation experiments during validation against FDA approved assay for Plasma Metanephrines

Experiments –
- Do we believe the MS results?
- Is there a calibration difference?
- Selectivity difference between assays?
- Did we use the same sample?
- Repeat assay samples?
- Stability issues and timing/storage?
- Do we expect equivalency anyway?

Was comparative result acceptable?

Answer: No

Solution: Repeat and include if comparative results is OK, or exclude with reasoning (we excluded, chromatogram and bias was the same, even on repeat in both assays).
Clinical Utility

Endocrinology
Cancer Biomarkers
Inborn Errors of Metabolism
Health and Wellness
Pain Management
Toxicology
Therapeutic Drug Monitoring
Learn all about the “how’s, the why’s, the when’s, and the what for’s” of mass spectrometric applications to medicine.

Keep up to date with the changing compliance and quality landscape of clinical diagnostics.

“This course should be on your bucket list!”

-David Herold, MD, PhD
USCD/VA Medical Center, San Diego