# *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*

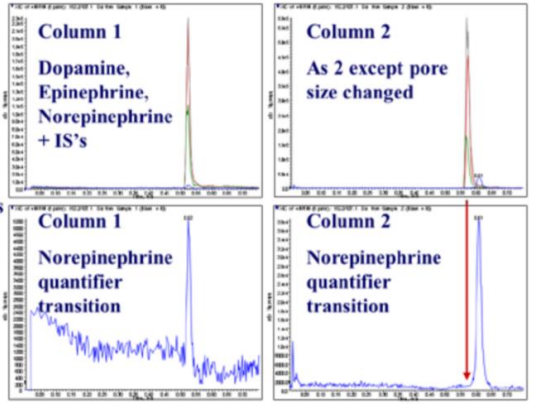
**Experiments –**

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?**

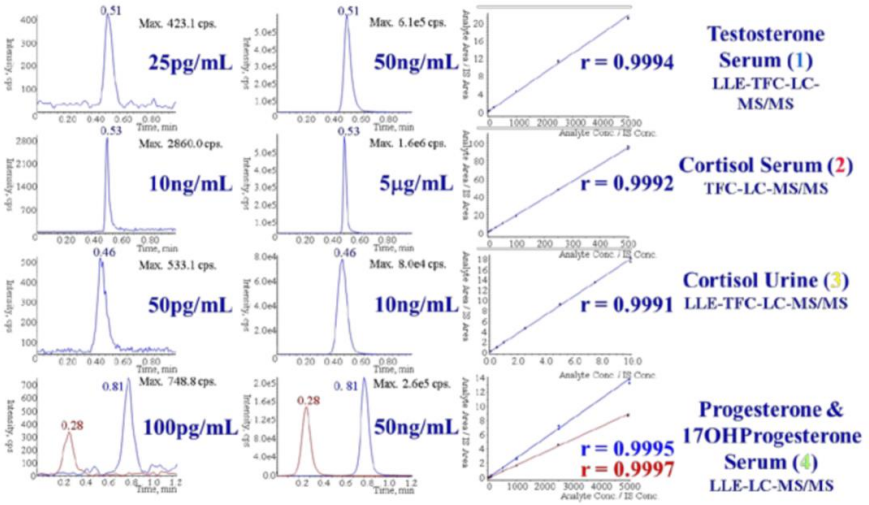
**Answer: NO**

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

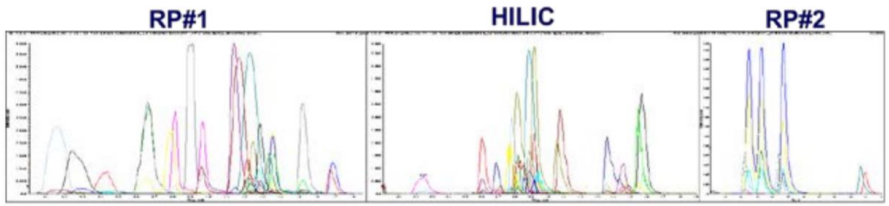



**Solution** – *Peak width of 1.2 seconds (unpredicted by Van deemter), changed pore size ONLY*

## Multiplexed Steroid Analysis




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




| | LLOQ | ULOQ | Low Plasma QC | Mid Plasma QC | High Plasma QC | Low Urine QC | Mid Urine QC | High Urine QC | |
|---|---|---|---|---|---|---|---|---|---|
| Intra-Assay Inaccuracy | 0%-13.6% | 0.6%-10.3% | N/A | N/A | N/A | N/A | N/A | N/A | n=20 |
| Intra-Assay Imprecision | 6.7%-12.8% | 2.3%-10.8% | 2.8%-11.9% | 3.0%-9.9% | 2.5%-10.9% | 4.7%-12.4% | 3.6%-11.3% | 5.4%-11.9 | n=20 |
| Inter-Assay Inaccuracy | 0.5%-8.5% | 0.8%-8.7% | N/A | N/A | N/A | N/A | N/A | N/A | n=20 |
| Inter-Assay Imprecision | 0.3%-4.6% | 0.1%-7.7% | 4.2%-11.8% | 5.7%-13.6% | 1.9%-14.2% | 7.0%-13.4% | 3.1%-12.6% | 2.3%-13.3% | n=20 |

**Quantitative**

| | | | |
|---|---|---|---|
| 1-Methyl-histidine | Cystine | Methionine | α-Aminobutyric acid |
| 3-Methyl-histidine | Glutamic acid | Ornithine | β-Alanine |
| Alanine | Glutamine | Phenylalanine | β-Aminoisobutyric acid |
| Alloisoleucine | Glycine | Proline | γ-Aminebutyric acid |
| Anserine | Histidine | Sarcosine | |
| Arginine | Homocitrulline | Serine | |
| Argininosuccinic acid | Homocystine | Taurine | |
| Asparagine | Hydroxylysine | Threonine | |
| Aspartic acid | Hydroxyproline | Tryptophan | |
| Carnosine | Isoleucine | Tyrosine | |
| Citrulline | Leucine | Valine | |
| Cystathionine | Lysine | α-Aminoadipic acid | |

**Qualitative**

Glycine-Proline
Saccharopine
Alpha-Acetyl-lysine
Epsilon-Acetyl-lysine
S-Sulfo-Cystine
D-ALA
FIGLU
Pyroline-5-carboxylate
Homoserine
Phosphoethanolamine
Homocarnosine
Orotic Acid

## 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*

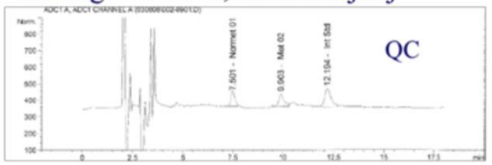
**Experiments –**

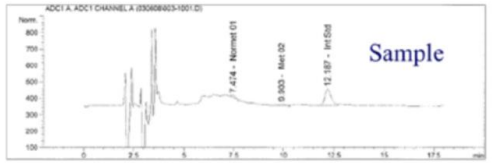
**Do we believe the MS results?**
**Is there a calibration difference?**
**Selectivity difference between assays?**
**Did we use the same sample?**
**Repeat assay samples?**
**Stability issues and timing/storage?**
**Do we expect equivalency anyway?**

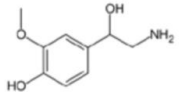
**Was comparative result acceptable?**

**Answer: No**

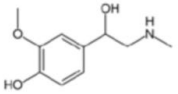
**Solution:** *Repeat and include if comparative results is OK, or exclude with reasoning (we excluded, chromatogram and bias was the same, even on repeat in both assays).*




Normetanephrine




Metanephrine

# ***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**