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

Instructors:
Russell Grant
Brian Rappold
Chris Shuford

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

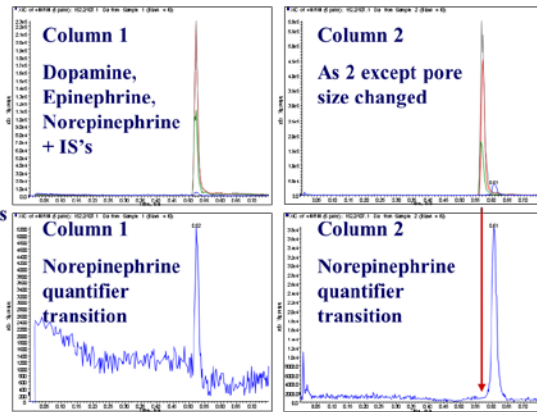# Real-World Examples and Trouble Shooting

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?

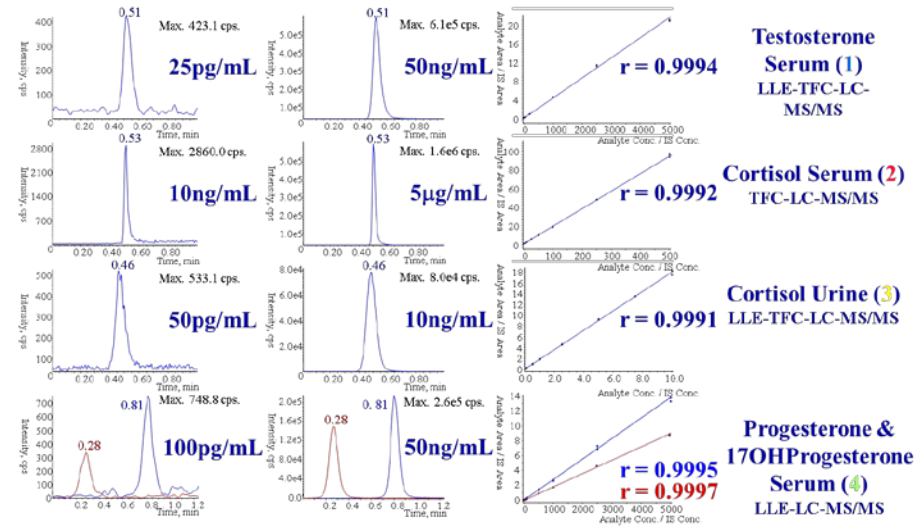**Is it really Norepinephrine even with transition ratios that match standards?**

Answer: NO

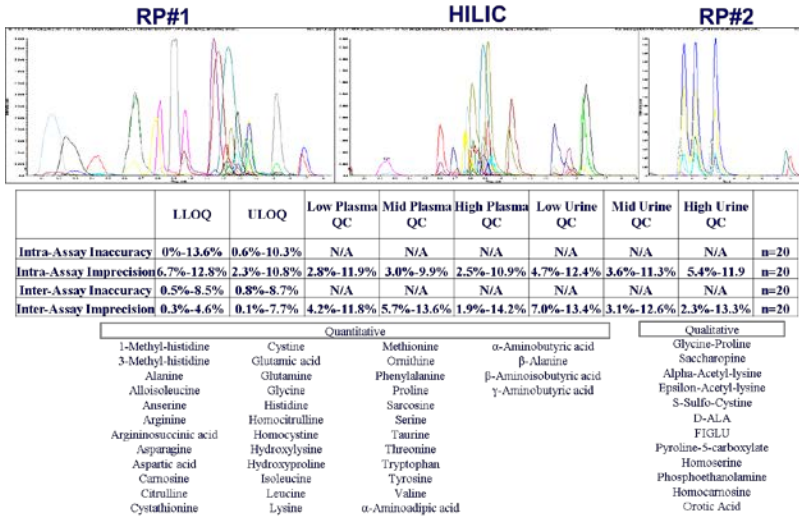**How do we know:** We changed LC separation (stationary phase), BUT sensitivity was not good enough

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

Column 1 — Dopamine, Epinephrine, Norepinephrine + IS's

Column 2 — As 2 except pore size changed

Column 1 — Norepinephrine quantifier transition

Column 2 — Norepinephrine quantifier transition

Multiplexed Steroid Analysis

25pg/mL Max. 423.1 cps.
50ng/mL Max. 6.1e5 cps.
r = 0.9994 **Testosterone Serum (1)** LLE-TFC-LC-MS/MS

10ng/mL Max. 2860.0 cps.
5µg/mL Max. 1.6e6 cps.
r = 0.9992 **Cortisol Serum (2)** TFC-LC-MS/MS

50pg/mL Max. 533.1 cps.
10ng/mL Max. 8.0e4 cps.
r = 0.9991 **Cortisol Urine (3)** LLE-TFC-LC-MS/MS

100pg/mL Max. 748.8 cps.
50ng/mL Max. 2.6e5 cps.
r = 0.9995
r = 0.9997 **Progesterone & 17OHProgesterone Serum (4)** LLE-LC-MS/MS

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

RP#1   HILIC   RP#2

	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				Qualitative
1-Methyl-histidine	Cystine	Methionine	α-Aminobutyric acid	Glycine-Proline
3-Methyl-histidine	Glutamic acid	Ornithine	β-Alanine	Saccharopine
Alanine	Glutamine	Phenylalanine	β-Aminoisobutyric acid	Alpha-Acetyl-lysine
Alloisoleucine	Glycine	Proline	γ-Aminobutyric acid	Epsilon-Acetyl-lysine
Anserine	Histidine	Sarcosine		S-Sulfo-Cystine
Arginine	Homocitrulline	Serine		D-ALA
Argininosuccinic acid	Homocystine	Taurine		FIGLU
Asparagine	Hydroxylysine	Threonine		Pyroline-5-carboxylate
Aspartic acid	Hydroxyproline	Tryptophan		Homoserine
Carnosine	Isoleucine	Tyrosine		Phosphoethanolamine
Citrulline	Leucine	Valine		Homocarnosine
Cystathionine	Lysine	α-Aminoadipic acid		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?
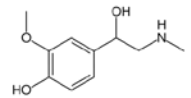**Do we expect equivalency anyway?**

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).*

QC

Sample
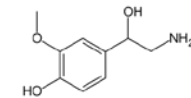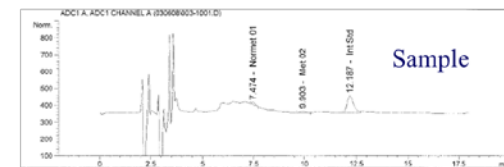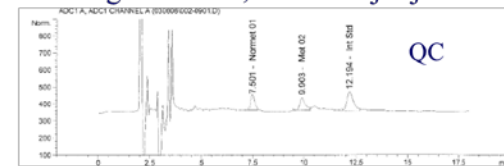
Normetanephrine   Metanephrine

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
UCSD/VA Medical Center, San Diego