

**Beyond Westgard Rules: Quality Control for Mass Spectrometry**

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**Assay Validation Criteria and Acceptance**

Validation Process	Materials Requirements and Acceptance Criteria
<b>Accuracy (Intra and Inter-assay)</b>	Min. 2 levels of spiked QC's including LLOQ and ULOQ; Min 20 measurements each in a single run and across 25 days. Bias % <15% or 20% @LLOQ
<b>Precision (Intra and Inter-assay)</b>	Min. 2 levels of spiked QC's including LLOQ and ULOQ; Min 20 measurements each in a single run and across 25 days. CV % 15%/20% @LLOQ
<b>Standard Curve Fit</b>	6-10 levels run in each assay, evaluated over 5 assays (days). R>0.995, Slope CV<5%
<b>Drug Selectivity</b>	Up to 350 potentially interfering endogenous and exogenous analytes spiked supraphysiological concentrations. Response < 20% LLOQ when spiked at Maximum Normal x10 ideal, minimally <20% LLOQ @ upper normal range.
<b>Materials Selectivity</b>	Blank matrix test 6 or more lots Response<20% LLOQ in 5 of 6.
<b>Matrix Interference</b>	Effect of lipid, Icteria and Hemolysis. Bias % <15%/20% @LLOQ Test anticoagulants Bias % <15%/20% @LLOQ Alternate sample types Deming regression 0.9-1.1, R >0.9 for same reference interval. Matrix effects (sample mixing) Bias<15%

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**Assay Validation Criteria and Acceptance**

Validation Process	Materials Requirements and Acceptance Criteria
<b>Carry-over</b>	After ULOQ - 3 runs including <20% LLOQ Stress test (10-100x ULOQ), how many for <20% LLOQ
<b>Dilutional Linearity</b>	5 Level sample mixing - Accuracy % 85% to 115% 1:1 - 1:50000 dilution - Accuracy % 85% to 115% Serial 100-10% dilution - Accuracy % 85% to 115%, (80/120%@LLOQ)
<b>Sample Stability</b>	All conditions tested from arm to final result Mean Bias < 15%/20% @ LLOQ, %CV < 15%/20% @ LLOQ, Deming regression 0.9-1.1, R >0.9
<b>Inter-assay Comparison</b>	Min 20 - External Disparate assays - For information only Internal assays same calcs - Deming regression 0.9-1.1, R >0.9 Same assay different system - Deming regression 0.95-1.05, R >0.95 Same assay Manual/Automation - Deming regression 0.95-1.05, R >0.95
<b>Reference Interval</b>	120 in each reference interval 20 (95% CI) for range transfer 1/20 outside existing range
<b>Transition ratio monitoring</b>	Bias from extracted calibrators in each batch <20% up to 3x LLOQ, < 15% after that for ratio>0.5
<b>Batch Size</b>	Maximum 184 samples prior to recalibrate, Bias % <15%/20% @LLOQ, % CV % <15%/20% @LLOQ, Carry-over <20% LLOQ Repeat samples n = 2 (first and 2nd plate) Deming regression 0.9-1.1, R >0.9

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**Assay Translation (R&D – Ops)**  
**(Assay Viability – QC Ranges Generation n=25 over 5 runs)**

<b>Verification Process</b>	<b>Materials Requirements and Acceptance Criteria</b>
<b>Materials Selectivity</b>	Blank matrix <20% LLOQ in 5 runs.
<b>Standard Linearity</b>	All Cals used, Accuracy % 85% to 115%, 80 – 120% @LLOQ and R>0.995, Slope CV<5%
<b>Carry-over</b>	After ULOQ – 3 runs including <20% LLOQ Stress test (10-100x ULOQ), how many for <20% LLOQ
<b>Precision (Intra and Inter-assay)</b>	Total 25 QC's over 5 runs %CV <15% or 20% @LLOQ
<b>Inter-assay Comparison</b>	Minimum n=20 per batch – Deming regression 0.90-1.00, R >0.90

**Do not use best technicians for this step**  
**Add multiple columns/materials lots /systems, time to emulate reality**

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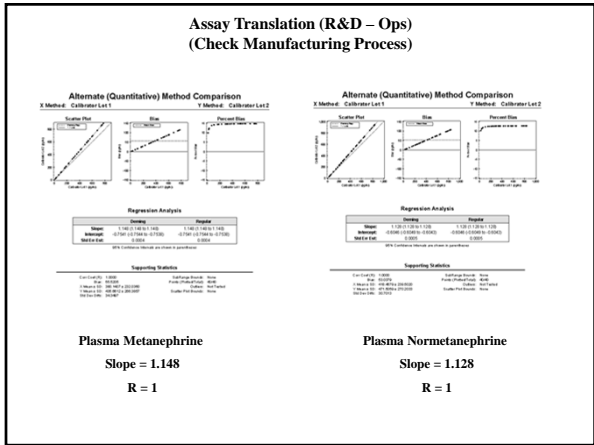
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**Internal QC for LC-MS/MS**

<b>LC System</b>	<b>Internal QC Action</b>
Power Failure	Shutdown (without UPS)
Leak detection	Shutdown*
Pressure beyond pumping	Shutdown
Air bubble	Shutdown or No monitoring
Column Degradation	No monitoring
Complete Sample volume injected	No monitoring
Injector blockage	No monitoring*
Contamination	No monitoring
<b>MS System</b>	<b>Internal QC Action</b>
Power Failure	Shutdown (without UPS)
Gas failure	Shutdown
Vacuum failure	Shutdown
Temperature effects	Shutdown – in extreme conditions
Contamination	No monitoring
Resolution change	No monitoring
Sensitivity drift	No monitoring
Selectivity	No monitoring

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**System Suitability Test (SST) – “The Doctor”**

Goal – System check prior to analysis to confirm appropriateness of test system for analysis/ or DRIFT

Design:

Neat solution stored under stable conditions – preferably at the assay LLOQ

Acquired correctly?

Retention time – 5% variance

Sensitivity – S:N >20:1

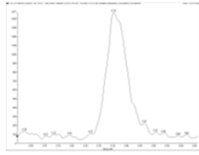
Peak shape – As 0.8-1.2

Multi-channel acceptance – column-column/channel-channel/system-system

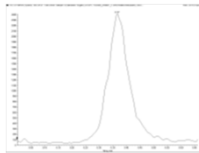
**SST is key part of troubleshooting experiments**

**Ignore the first (or second) injection**

Urine Cortisol SST



Urine Cortisol LLOQ




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**HPLC Pressure Trace – “The Heartbeat”**

Goal – Pre-batch and per-injection check of appropriate LC performance

Design:

SOP Example – Annotate an acceptable pressure trace

Pressure profile – Start and overall maximum pressure

Changes in pressure profile –

Air bubbles in A or B

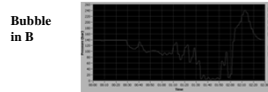
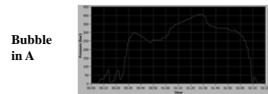
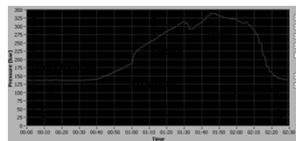
Dead volume differences

Column dimensions change

Full injection

Injector/filter/column/lines blockage

Post column blockage (bypass/source probe)




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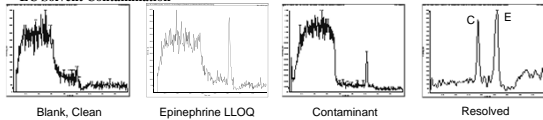
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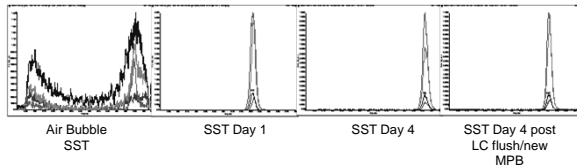
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**Internal QA – Pre-Batch**

LC Solvent Contamination



Air Bubble/Column or MP Degradation/Injector blockage




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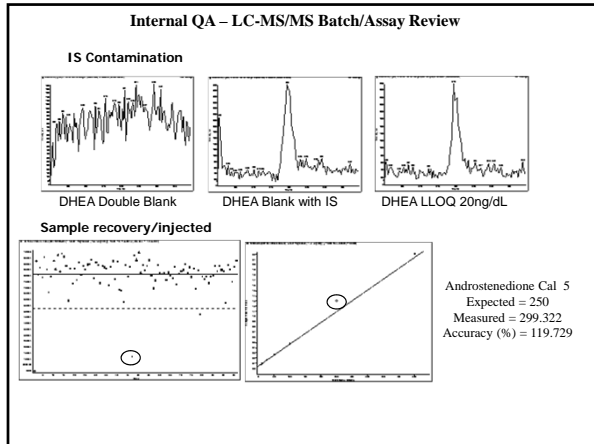
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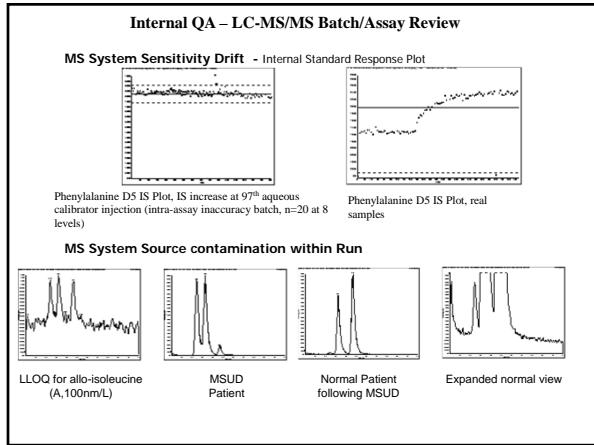
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**Internal QA – LC-MS/MS Batch/Assay Review**  
**Matrix Effects in Individual samples – Signal:Noise**

Sample	Analyte	Conc	RT	Analyte Peak Area	IS	Response	Calc conc	Ion Ratio	Ratio	Flag?
Std D	D3	200	2.84	13902	12597	1.10	201.1	1.4	NO	
Std C	D3	100	2.82	5210	10349	0.50	99.8	1.3	NO	
Std B	D3	30	2.88	2128	11640	0.18	33.3	1.4	NO	
Std A	D3	1	2.87	249	10274	0.02	1.1	1.4	NO	
Ctrl HI	D3	2.88	808	13878	0.06	10.7	1.4	NO		
Ctrl LO	D3	2.88	3190	12186	0.26	48.4	1.4	NO		
M64598	D3	2.89	2809	8234	0.34	63.0	1.3	NO		
M67899	D3	2.86	1408	13192	0.11	19.7	1.5	NO		
M70133	D3	2.84	1504	13469	0.11	20.6	1.5	NO		
M70134	D3	2.82	1443	10960	0.13	24.3	1.2	NO		
M70135	D3	2.90	1703	12058	0.14	26.1	1.3	NO		
M71222	D3	2.80	1774	13004	0.14	25.2	1.4	NO		
M72090	D3	2.84	1932	11747	0.16	30.4	1.4	NO		
T80031	D3	2.83	2034	2312	0.89	162.9	1.4	NO		
T80100	D3	2.83	2178	13075	0.17	30.5	1.3	NO		
T81070	D3	2.89	4508	12578	0.36	65.7	1.2	NO		
T90909	D3	2.83	3488	11378	0.31	56.7	1.4	NO		
T91002	D3	2.80	2912	12790	0.23	42.1	1.4	NO		
T91114	D3	2.84	2600	12610	0.21	38.1	1.5	NO		

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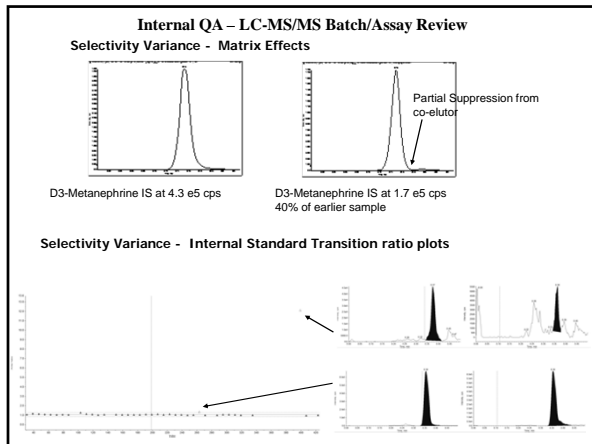
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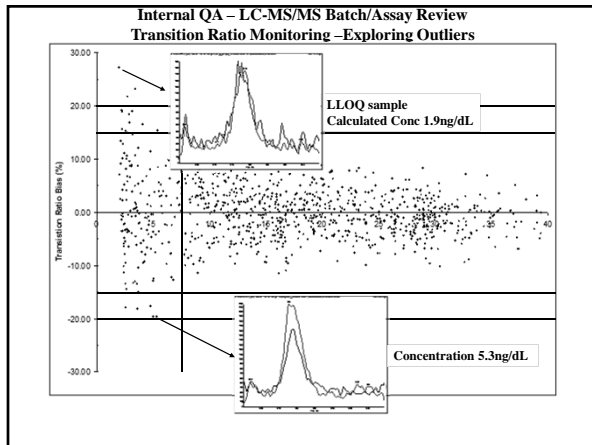
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**Internal QA – LC-MS/MS Batch/Assay Review**  
**Transition Ratio Monitoring –Exploring Outliers**

Sample	Analyte	Conc	RT	Analyte Peak Area	IS	Response	Calc conc	Ion Ratio	Ratio Flag?
Std D	D3	200	2.81	12870	12370	1.04	198.9	1.5	NO
Std C	D3	100	2.82	5990	12335	0.49	100.1	1.5	NO
Std B	D3	30	2.88	2288	13237	0.17	32.9	1.5	NO
Std A	D3	1	2.88	252	13704	0.02	0.9	1.4	NO
Ctrl HI	D3	2.90	780	13109	0.06	11.6	1.4	NO	
Ctrl LO	D3	2.86	3501	13932	0.25	48.7	1.5	NO	
W54634	D3	2.83	1650	13081	0.13	24.5	1.5	NO	
W58132	D3	2.86	1249	12703	0.10	19.1	1.5	NO	
W66023	D3	2.89	1641	13211	0.12	24.1	1.5	NO	
W62743	D3	2.81	2423	12636	0.19	37.2	1.5	NO	
W66817	D3	2.86	2208	13543	0.16	31.6	1.5	NO	
H66438	D3	2.83	2359	12163	0.19	37.6	1.4	NO	
H62633	D3	2.81	2057	13559	0.15	29.4	1.5	NO	
H78948	D3	2.82	1593	12762	0.12	24.2	1.4	NO	
H78388	D3	2.82	2836	12824	0.22	42.9	1.4	NO	
H74054	D3	2.87	1257	12469	0.10	19.6	1.5	NO	
H87727	D3	2.67	1362	12944	0.11	20.4	1.9	YES	
H89927	D3	2.85	1851	12528	0.15	28.7	1.4	NO	
H88721	D3	2.89	2731	13284	0.21	39.9	1.4	NO	

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### External QC 3-Plex Linked Analytes Plasma Metanephrines The importance of Automation

Manual Preparation First Run with SPE

Automated First Run With SPE

Recovery Variance ADDS to Imprecision which makes Westgard Rules Impossible to apply  
Use Automation OR Train Technicians VERY well

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### External QC - Calibrator Stability

Cocaine Profile  
LOQ (1 ng/mL)

Cocaine Profile  
ULOQ (1000 ng/mL)

Cocaine Stability

Plasma QC stability at 2-8C inc 1% NaF  
Need Alternate Matrix to assess Calibrator Stability

Cocaine Profile  
Plasma QC1 (5 ng/mL)

Cocaine Profile  
Urine QC  
(100-110 ng/mL)

Cocaine Profile  
Organic QC  
(105-110 ng/mL)

Cocaine Profile  
Blood QC  
(90-105 ng/mL)

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### Column Verification

#### CAP Checklist, Gas Chromatography and High Performance Liquid Chromatography Section

QHM 1650	Column Verification	Phase 3
	New columns are verified for performance before use.	
	Evidence of Compliance:	
	<input checked="" type="checkbox"/> Written procedure for column verification AND	
	<input checked="" type="checkbox"/> Records of column verification	

**“The 3 R’s”:** Verifiable Column Performance Characteristics

**Retention** – Are all analytes properly adsorbed to the new stationary phase

**Resolution** – Are all analytes exhibiting appropriate desorption from the new stationary phase

**Response** – Are any components of the new column affecting MS ionization efficiency

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**When Accuracy based QC isn't accurate**

**External Accuracy based mixture (>50 analytes) in Phenol/water**

**Supra-physiological ~250uM**

**Measure Neat (no IS),  
Neat diluted (class A)**

**Determine how to deploy "accuracy" based materials that do not represent the clinically observed concentrations of mixtures**

Neat Pickering QC No IS added

10 x Diluted Pickering QC + IS

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**Inter-Laboratory Correlation LabCorp and St Pauls, BC**

**Regression Plot**

**Difference Plot**

**Concentrations < 45ng/dL**

**N= 52**

**Intercept: -0.096**

**CI Intercept: [-0.557,0.488]**

**Slope: 0.985**

**CI Slope: [0.954,1.014]**

**R-squared: 0.990513**

**Mean Difference: -2.49%**

**SD Difference: 5.304%**

**Acknowledgement: Dr Daniel Holmes,  
Grace Van Der Gugten**

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**Automated Data review**

**If [examined value] exceeds [a threshold], then flag with [text string].**

<b>Calibration Concentration Deviation</b>	deviates from nominal by N	<b>Ion Ratio Deviation</b>	Quant/Quant deviates from expected by N
<b>Calibration No Intercept</b>	response undefined on a non-linear calibration curve	<b>Ion Ratio Peak Missing</b>	Quant, no Quant or Quant, no Quant
<b>Calibration Regression Failed</b>	calibration curve uncalculatable	<b>Peak Fit Quality</b>	alignment of peak with data < N
<b>Calibration Standards Excluded</b>	more than N standard samples not used for calibration curve	<b>Peak High Signal No Peak</b>	no peak and observed intensity > N
<b>Subsequent to Carryover</b>	N samples after a possible carryover	<b>QC Concentration Deviation</b>	deviates from nominal by N
<b>Dilution Required</b>	concentration > ULOQ	<b>QC Exceeds Standard Deviation</b>	deviates from nominal by N SD
<b>Overdiluted</b>	present and concentration < LLOQ and dilution factor > 1	<b>QC Negative Control</b>	nominal = 0 and calculated > N
<b>Present but Below LLOQ</b>	present and concentration < LLOQ	<b>QC Flag Positive Unknowns</b>	present and unknown sample when any QC flag raised
<b>Contamination of Blank</b>	blank and concentration > N	<b>RT Quant Qual Difference</b>	Quant RT - Qual RT   > N
<b>Internal Standard Area Deviation</b>	deviates from expected by N	<b>RT Relative Deviation</b>	Quant/IS RT deviates from expected by N
		<b>RT Shift From Standards</b>	Quant RT deviates from expected by N

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### Acknowledgements

Patricia Holland, Matt Crawford, Stacy Dee, Mary Morr, Dr Marcia Eisenberg, Dr Walt Chandler

Brian Rappold

Randy Julian

Dr Andy Hoofnagle

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