

## Overview

Bioanalytical method validations require the preparation of both routine and specialized samples. However, the process is similar from one molecule to the next, and much of the work can be handled robotically if terms and procedures can be standardized.

In pilot experiments with a Hamilton Star liquid handler, we were able to show that a robotic system could prepare method validation and sample analysis runs with significantly better precision and accuracy than human analysts, and therefore our goal was to devise an instrument qualification plan that would validate this approach for general laboratory use.

An instrument qualification plan was composed and executed that confirmed the reliability and accuracy of each sample preparation step. By using standardized terms for each type of sample, the system was able to process the Watson worklist and then construct the various sample types on deck with a high degree of assurance. Completion of the instrument qualification plan has permitted the Hamilton Star system to be used in our laboratory for both regulated and nonregulated method validation and sample analysis experiments.

## Qualification Strategy

The system as installed had passed the manufacturer's basic installation qualification (IQ) for precision and accuracy in aspiration and dilution, pressurization checks, and mechanical alignment. To qualify the system for our use, we significantly extended the testing to prove that AIT's program code (based on the Hamilton Venus 4.2 language) could provide acceptable validation and sample analysis data.

Our testing *did not explicitly verify* that each of the dozens of commands in the Venus language performed exactly as specified or that nonsense inputs were prevented. Instead, we grouped such commands by their overall purpose into modules that could be verified holistically (e.g., dilute a working standard to create a set of 8 spiking solutions over 3 orders of magnitude- then verify overall accuracy, linearity, and precision by LC-MS/MS analysis).

To perform these holistic tests, we used a well-understood manual 96-well method for methadone and its EDDP metabolite in human plasma. Four runs were created to test the processing of every possible sample type, run length, errors in setup, and mechanical faults. The qualification also included the testing of user role assignments and file security measures to protect methods against unauthorized or inadvertent change or deletion.

## Methods

### Key Instrumentation and Software

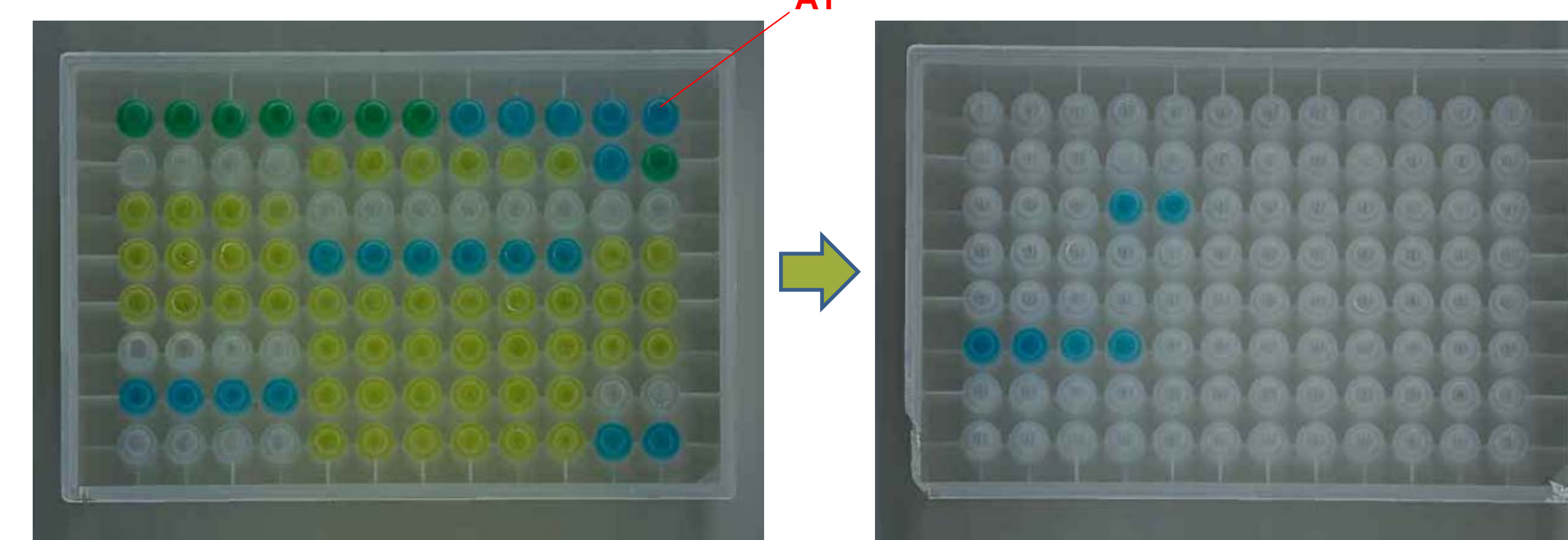
- Hamilton Star Liquid Handler with Venus 4.2 Command Software
- Thermo Scientific TSQ Vantage mass spectrometer
- Watson LIMS v 7.4 SP2 and TSQ Module v 1.0
- IDBS E-WorkBook 2010 v 8.3.1

## Key Experiments

	Run 1	Run 2	Run 3	Run 4
Prepare set of neat spiking solutions, using 8 serial dilutions	X	X		X
Prepare fresh set of 8 calibration stds from blank matrix and spiking solutions (parallel dilutions)	X	X		X
Transfer control samples and carryover samples	X	X	X	X
Prepare single plate run	X			X
Add internal std solution using 96 head		X	X	X
Test for missing or extra samples on deck compared to Watson runlist	X			X
Test bar code identification, stop for unreadable barcodes	X			
Create diluted samples according to dilution factor in Watson runlist			X	X
Create runs of >200 samples over 3 plates		X	X	
Test liquid level detection (and liquid following) during aspiration	X			
Assess transfer integrity (dripping)		X		
Prepare wells for recovery, matrix factor, and system check samples according to sample descriptor in Watson run list	system check only	system check only	all	system check only
Transfer QC, validation samples, selectivity, and stability samples according to barcode	VS only	VS only	all	QC only
Extraction, LC-MS/MS analysis, Watson processing	X	X		X
Visual analysis of solutions dispensed			X	

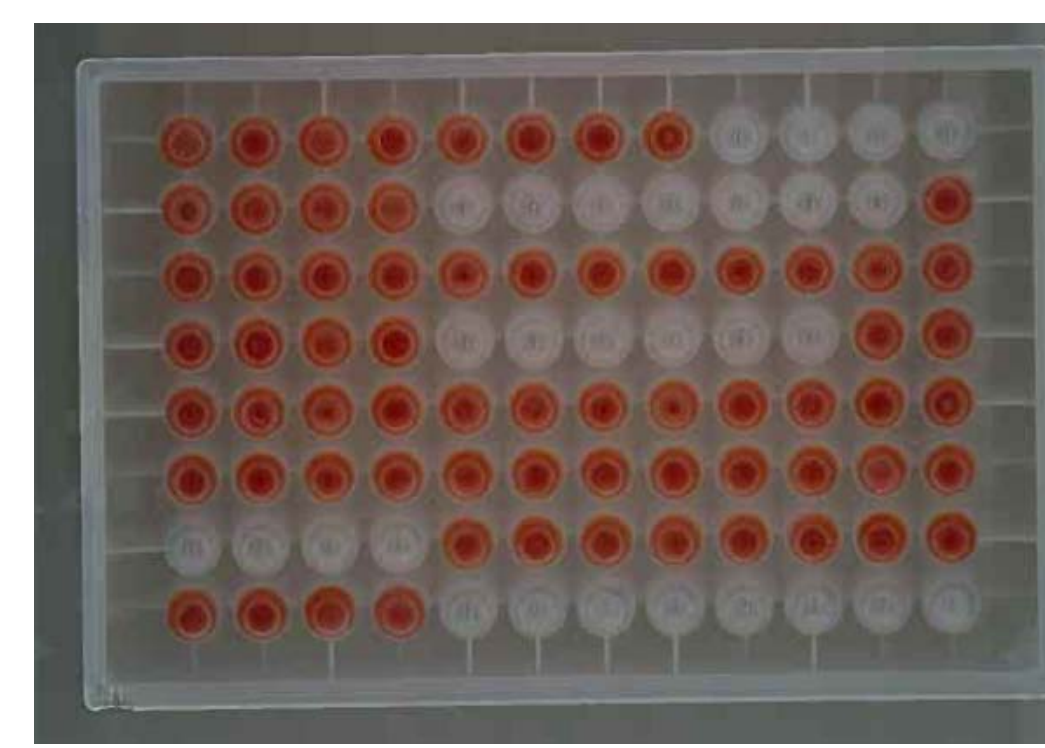
Figure 1. Hamilton runlist (from Watson) for the first 24 samples of Run 3. Bottom views of plate 1 after 4 steps in the processing are shown below.

Sample Name	Source Rack	Source Tube	Dest Plate	Dest Well	Vol Sample	(Istd)	(Surro.Istd)
System Check	Blank_Plasma_Trough	7	Plate_01	A1	100		X
System Check	Blank_Plasma_Trough	8	Plate_01	A2	100		X
Control Negative	Blank_Plasma_Trough	9	Plate_01	A3	100		X
Control Negative	Blank_Plasma_Trough	10	Plate_01	A4	100		X
Control Positive	Blank_Plasma_Trough	11	Plate_01	A5	100	X	
CS 8	Overhead_samples	17	Plate_01	A6	100	X	
CS 7	Overhead_samples	16	Plate_01	A7	100	X	
CS 6	Overhead_samples	15	Plate_01	A8	100	X	
CS 5	Overhead_samples	14	Plate_01	A9	100	X	
CS 4	Overhead_samples	13	Plate_01	A10	100	X	
CS 3	Overhead_samples	12	Plate_01	A11	100	X	
CS 2	Overhead_samples	11	Plate_01	A12	100	X	
CS 1	Overhead_samples	10	Plate_01	B1	100	X	
Carryover	Blank_Plasma_Trough	1	Plate_01	B2	100		X
SELECTIVITY LOT A	Tube_Rack	10005	Plate_01	B3	100		X
SELECTIVITY LOT B	Tube_Rack	10006	Plate_01	B4	100		X
SELECTIVITY LOT C	Tube_Rack	10007	Plate_01	B5	100		X
SELECTIVITY LOT D	Tube_Rack	10008	Plate_01	B6	100		X
SELECTIVITY LOT E	Tube_Rack	10009	Plate_01	B7	100		X
SELECTIVITY LOT F	Tube_Rack	10010	Plate_01	B8	100		X
LLOQ VS	Tube_Rack	10011	Plate_01	B9	100	X	
LLOQ VS	Tube_Rack	10011	Plate_01	B10	100	X	
LLOQ VS	Tube_Rack	10011	Plate_01	B11	100	X	
LLOQ VS	Tube_Rack	10011	Plate_01	B12	100	X	
LLOQ VS	Tube_Rack	10011	Plate_01	C1	100	X	
LLOQ VS	Tube_Rack	10011	Plate_01	C2	100	X	
LOW VS	Tube_Rack	10012	Plate_01	C3	100	X	
LOW VS	Tube_Rack	10012	Plate_01	C4	100	X	
LOW VS	Tube_Rack	10012	Plate_01	C5	100	X	
LOW VS	Tube_Rack	10012	Plate_01	C6	100	X	
LOW VS	Tube_Rack	10012	Plate_01	C7	100	X	
LOW VS	Tube_Rack	10012	Plate_01	C8	50	X	
LOW VS HEMOLYSIS	Tube_Rack	10013	Plate_01	C9	50	X	
LOW VS HEMOLYSIS	Tube_Rack	10013	Plate_01	C10	100	X	
LOW VS HEMOLYSIS	Tube_Rack	10013	Plate_01	C11	100	X	
LOW VS CO-ADMIN A	Tube_Rack	10014	Plate_01	C12	100	X	



Blue: system checks, control ± ISTD, recovery samples, carryover samples.  
Green: calibration standards  
Clear: QC samples (by barcode)  
Yellow: validation samples (by barcode)

Blue: makeup volume of control matrix for dilutions



Orange: internal standard

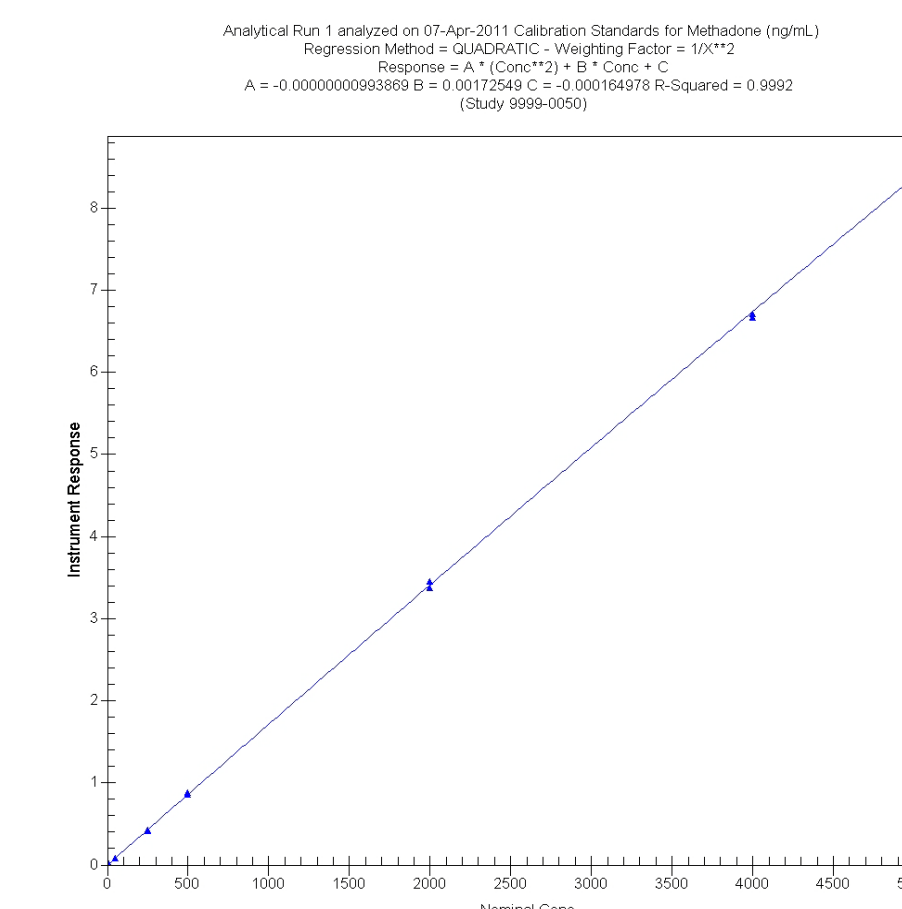


Red: surrogate internal standard

Figure 2. Methadone calibration and validation sample results from Run 1 of Instrument Qualification

	CS 8	CS 7	CS 6	CS 5	CS 4	CS 3	CS 2	CS 1
Theor. Conc.	5	10	50	250	500	2000	4000	5000
Found Conc.								
#1	4.82	9.58	49.8	243	500	2020	3980	5050
#2	5.14	10.6	50.6	249	510	1980	3960	5010
Mean	4.98	10.1	50.2	246	505	2000	3970	5030
%Theoretical	99.6	101	100.4	98.4	101	100	99.3	100.6
%Bias	-0.4	1	0.4	-1.6	1	0	-0.8	0.6
n	2	2	2	2	2	2	2	2

	LLOQ VS	LOW VS	MIDDLE VS	HIGH VS
Theor. Conc.	5	15	375	3750
Found Conc.				
#1	5.43	15.7	374	3770
#2	5.23	15.7	371	3750
#3	5.33	15.9	377	3900
#4	5.29	16.1	392	3820
#5	5.47	15.2	390	3740
#6	5.21	15.7	379	3740
Mean	5.33	15.7	381	3790
S.D.	0.105	0.299	8.6	63.1
%CV	2	1.9	2.3	1.7
%Theoretical	106.6	104.7	101.6	101.1
%Bias	6.6	4.7	1.6	1.1
n	6	6	6	6



## Results and Conclusions

The test scripts evaluated the performance of the system towards recognizing the requirements of each Watson worklist. System-generated standard curves covering 3 orders of magnitude demonstrated mean bias less than 5%, with most values less than 2%. Barcoded sample positions were recognized and aspirated and/or diluted correctly for transfer to plates. Quality control pools were measured with CV's ranging from 3% to < 1%. Carryover and control negative samples were never contaminated in any machine transfers. Analytical runs of up to 250 samples were interpreted and processed correctly, whether for validation or sample analysis.

Automated processing of samples on the Hamilton system gave results that were more accurate and precise than manual means. The test scripts provided a reasonable confidence that the system would improve lab efficiency and eliminate inter-analyst dependencies on assay performance. The operating program permitted the construction of analytical runs without human intervention.