
Automation in Drug Development: Challenges and Rewards

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Outline

- Historical perspective
- Why Automate ?
- Criteria for Automation
- Validated Development Assays
- Challenges and Rewards
- Conclusions

Historical perspective

- Traditionally, robotic systems have been used in the Drug Discovery environment as a High Throughput Screening (HTS) tool.
- Automated systems are now being routinely used in preclinical and clinical labs to increase throughput and overall assay quality by improving precision and accuracy.
- Automation of routine procedures allows for re-designation of resources, thus leading to improvement in efficiency.
- At Boehringer-Ingelheim Pharmaceuticals Inc., the SciCLONE workstation is currently being used to automate a variety of enzymatic reactions.

Why Automate ?

- Increase productivity.
- Minimize analyst to analyst variability.
- Standardize current assays.
- Allow laboratory scientists to carry out different assays (open access capabilities).
- Improve overall assay quality by improving precision and accuracy.

Current Automated Assays in Drug Metabolism

- Probe substrate K_m and V_{max} determinations
- Metabolic stability
- IC_{50} determinations
- K_i determinations
- Inactivation
- Reaction phenotyping
 - Chemical inhibitors
 - Recombinant CYP450

SciCLONE Workstation



Criteria

- A thermal block to maintain constant temperature.
 - Accurate temperature ($37^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$) maintenance required to carry out enzymatic assays.
 - Mécour thermal block comparable to a circulating water bath
- A flexible deck layout.
 - Accommodate a variety of consumables.
- Carry out assays using timed events with a high degree of accuracy

Mécour Hot Block



Criteria

- A 96 well head for maximum sample throughput
 - Flexible programming features
- Device that would load/unload consumables to and from the deck as needed
 - Assays that use a large amount of tips and other consumables
 - Limited deck positions
- Open access capability
 - Train colleagues to use the system for their assay(s) of choice

Assay Validation in Drug Metabolism



- To assure that *in vitro* experiments are performed with the utmost confidence in the methods used and data obtained.
- Determine analytical variability within an assay.
 - 3 separate days of validation including one day for process stability.
 - Each run/day included 2 separate STD (standard) curves (8 points each), 5 QC (quality control) concentrations and 6 replicate of each QC concentration.
 - Criteria:

	Coefficient of Variation	Relative Error
LLOQ QC	(%CV) ± 20	(%RE) ± 20
5 additional QCs	± 15	± 15

Bioanalytical Validation in Human Liver

Microsomes

Precision (%CV) Analysis



	QC1 ULOQ	QC2 80% ULOQ	QC3 Mid QC	QC4 3.2 X LLOQ	QC5 LLOQ	STD Curve Range (µM)
CYP1A2 Acetaminophe	9.26	8.24	5.02	4.84	4.46	0.00293-0.750
CYP2C9 4'-OH- Diclofenac	12.5	8.56	6.85	6.31	6.42	0.00391-0.500
CYP2C19 4'-OH- Mephenytoin	12.1	7.84	6.15	5.78	4.77	0.00195-0.250
CYP2D6 1'-OH- Bufuralol	9.26	8.24	5.02	4.84	4.46	0.00117-0.150
CYP2D6 Dextrorphan	10.4	4.93	5.40	4.50	8.90	0.000195- 0.250
CYP3A4 1'-OH- Midazolam	12.5	11.5	7.07	7.30	6.25	0.00293-0.375
CYP3A4 6-β-OH- Testosterone	9.25	6.44	6.38	4.92	6.14	0.0156-2.00

Bioanalytical Validation in Human Liver

Microsomes

Accuracy (%RE) Analysis



	QC1 ULOQ	QC2 80% ULOQ	QC3 Mid QC	QC4 3.2X LLOQ	QC5 LLOQ
CYP1A2 Acetaminophen	-2.29	0.06	-3.23	-5.95	6.62
CYP2C9 4'-OH-Diclofenac	-6.68	0.32	-6.14	-3.56	-4.26
CYP2C19 4'-OH- Mephenytoin	-4.39	4.37	2.04	5.78	-4.18
CYP2D6 1'-OH-Bufuralol	-4.90	2.14	-0.89	-8.89	-9.07
CYP2D6 Dextrorphan	-4.25	-0.40	-1.69	-4.42	-7.60
CYP3A4 1'-OH-Midazolam	-0.17	3.55	2.38	-3.06	-3.07
CYP3A4 6-β-OH- Testosterone	-4.00	1.63	2.83	3.59	3.24

Assay Validation in Drug Development

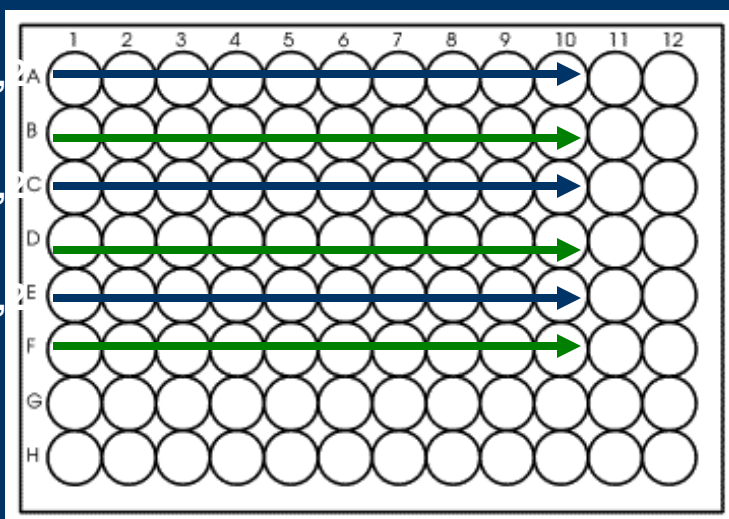
- Set up acceptance criteria, which will allow us to define acceptable ranges for enzymatic assays (pass/fail).
 - New stock solutions prepared daily
 - 1 experiment/day for 5 days
 - 2 protein concentration
 - 10 substrate concentrations (highest substrate $10 \times K_m$)
 - 3 time points
 - 2 standard curves (beginning and end of assay)
 - Quality control samples (dispersed throughout the run)

Sample and STD/QC Plates following K_m and V_{max} Assay completion

Protein #1,
5 min

Protein #1,
10 min

Protein #1,
15 min



Assay plate

- 3 time points
- 2 protein concentrations
- 10 substrate concentrations

STD #1

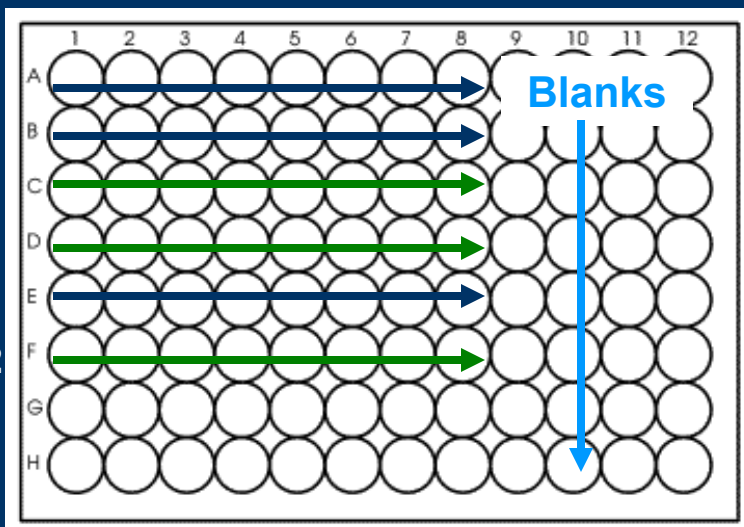
STD #1

STD #2

STD #2

QC #1

QC #2



STD and QC plate

- Two STD (8 points) curves per protein concentration.
- 6 Sets of quality control samples per protein concentration

K_m and V_{max} Parameters for the 6 Probe Substrates in Human Liver Microsomes

Probe Substrate	CYP450	K_m (μM)	%CV	V_{max} (pmol/min/mg)	%CV
Phenacetin	1A2	42	3.8	622	4.9
Diclofenac	2C9	4.3	21	3366	9.8
(S)-Mephenytoin	2C19	30	6.7	215	13
Bufuralol	2D6	26	11	355	6.0
Dextromethorphan	2D6	6.6	14	281	7.1
Midazolam	3A4	2.7	5.9	1021	8.8
Testosterone	3A4	77	6.0	5905	7.3

n = 5 determinations/CYP450

CYP450 Assay Challenges

- Biohazard/biological samples
 - Ensure adequate tips on deck
 - Trash box for containment; no chute through deck
 - Disposable tips
- Ensure precise timing and temperature of reactions because rates are measured.
 - Minimize variability
- Organic solvent evaporation
 - All mother plates of substrates, inhibitors are prepared and diluted with organic solvent.
 - Plates are covered with lids to avoid solvent evaporation
- Microsomes
 - Sensitive to temperature changes
 - Need to be handled gently and cannot be pipetted or mixed vigorously

Hardware and software challenges

- The original thermal block purchased from the manufacturer did not meet our specifications.
 - Purchased block from Mécour.
- The original deck was not solvent resistant.
 - Deck was subsequently anodized and is now solvent resistant.
- The SciCLONE head picks up tips along with the tip box, random problem.
 - The head was replaced with a new head (4 pins on each corner).

Hardware and Software Challenges

- Eight way syringe manifold pops out whenever the manifold arm comes down. The manifold is currently taped.
 - The manifold was replaced and is no longer taped
- Complex protocols have to be written if the 96 well head is not used as one unit.
- Direct assay transfer (manual to automated system) is not always feasible.
- Hard drive failure. Image current hard drive to avoid loss of protocols and peripheral device configurations.

Rewards

- Automation minimizes inter day assay variability and between analyst assay variability.
- Increased assay throughput by at least 2-fold.
- The SciCLONE can pipette low volumes (5 μ L) accurately and precisely. (Validated through calibration every 6 months)
- Reactions are all started and terminated at the same time with the use of the 96 well head.
- Programmed as an open access system
- Increased precision and accuracy for all *in vitro* assays.

Conclusions

- Traditionally, robotic systems have been used in the Drug Discovery environment as a High Throughput Screening (HTS) tool.
- In the areas of Preclinical and Clinical Development, automated systems are now being routinely used to increase throughput and overall assay quality by improving precision and accuracy.
- Automation of routine procedures allows for re-designation of resources, thus leading to improvement in efficiency.
- Automation has improved the quality of data generated from complex *in vitro* assays.
- Throughput with each *in vitro* assay has increased by at least