The application of Design of Experiment and the dragonfly® discovery to miniaturise, automate and accelerate assay optimization

L. Scott1, P. Craggs1, M. Pemberton1, R. Lewis2, P. Beech2, E. Schwam2, G. Cochrane2, J. Jenkins2, M. Leveridge1

1GlaxoSmithKline, Platform Technology & Science, Screening Profiling & Mechanistic Biology, Stevenage, UK / 2TT Labtech Ltd, Melbourn Science Park, Herts, UK

Correspondence: peter.d.craggs@gsk.com

Introduction

Traditional, iterative assay development, though employed and respected throughout the pharmaceutical industry, can be a long, sometimes challenging and cumulatively expensive process. Due to these long and often challenging assay development efforts there is renewed interest in employing methodologies, such as Design of Experiments (DoE) using JMP software, to apply a more statistical, streamlined approach to reduce timelines. Automation to carry out complex DoE experiments has always been a limiting factor, both in terms of speed and cost. The dragonfly® discovery from TTP Labtech, currently in early development, is a rapid and reliable low volume liquid handler that utilises true positive displacement technology to enable the dispensing of all aqueous solutions and substrates from 200nl through to 4ml. The dragonfly® discovery's plate-map file format made it simple to convert and import JMP DoE files and subsequently execute these experimental designs in all microplate types, including 1,536 well plates.

Here we present fluorescence polarization (FP) and time-resolved fluorescence resonance energy transfer (TR-FRET) assay development and screening data for a bromodomain (Brd) binding interaction. DoE and a prototype of the dragonfly® discovery were employed to develop these assays in 1,536 well microplates in rapid time compared to traditional methods. This was achieved without the use of any manual pipetting and 384 well microplates, in addition to a significant reduction in reagent requirements due to low liquid handler dead volumes. A further advantage of using the dragonfly® discovery for low volume, 1,536 well microplate assay development was that we were able to utilise the same liquid handling technology to then screen in excess of 40,000 compounds.

This combination of Design of Experiments and dragonfly® discovery demonstrates that it is possible to reduce assay development cycles times, in addition to removing well known assay bottlenecks associated with the transfer of subsequent assays into high throughput screening.

Design of Experiment

• Traditional assay development
  - Change one experimental factor at a time and keep all others fixed
  - Information on the factor you are changing but no interaction effects between factors measured

• Design of Experiment (DoE) assay development
  - Measures effects of individual experimental factors and the interaction between factors using statistical models

There is now renewed interest in DoE for assay development as a tool for reducing assay cycle times and aiding the investigation of multifaceted problems of increasingly complex systems.

Key Features of the dragonfly® discovery (DFD):
• True positive displacement technology
• Able to dispense a wide range of fluids and volumes, upwards from 200nl, with no changes to the instrument settings
• Accurate and reliable, 95% CV for the majority of liquid classes in all microplate densities
• Non-contact dispensing
• Minimal dead volume reducing reagent wastage
• Disposable tips and reservoirs
• 96-, 384- and 1,536-well microplate capability
• Simple file format allows third party software integration

FP Screening Data

Data Statistic

Mean (nl) 55 ± 0.5

Stdev deviation (nl) 5 7 8.6

% Inhibition cut-off 10 16 19.5

IC50 (nM) 0.5 2.4 7.1

Mean Z’ 0.7 0.85 0.76

Excellent assay performance - low interference and identification of known Brd inhibitors

Conclusions

Successful implementation of Design of Experiment and the dragonfly® discovery into a new mode of assay development and validation
• Expansion of high throughput technology - reduction in assay time, labour and reagent requirements through full 1536-well development
• Increased efficiency - maximising and prioritising data collection and reducing cycle times by combining Design of Experiment and the dragonfly® discovery
• Increased reliability - the ability of the dragonfly® discovery to dispense a wide range of solutions and volumes rapidly and accurately
• Decreased assay transfer ‘bottlenecks’ - utilisation of the same liquid handling technology throughout the assay development and screening process
• High quality screening data - Bird FP assay data demonstrates good assay quality and reproducibility