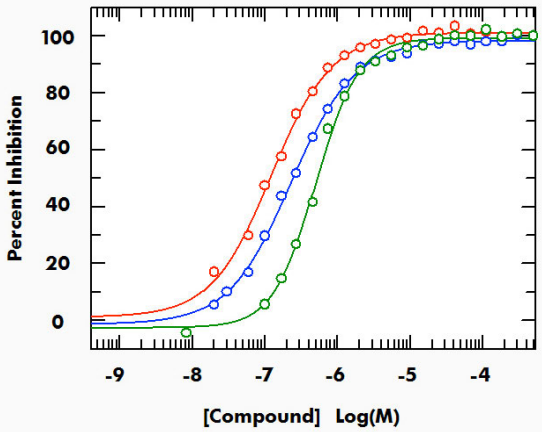

Reinventing drug titration for IC_{50} determinations

HP D300 Digital Dispenser

Drawbacks of traditional serial dilutions

The traditional serial dilution process for IC_{50} determinations of small molecules has numerous drawbacks, including:

- being limited to working in the microliter range;
- superfluous compound consumption;
- high risk of (cross-)contamination;
- inter-well dependencies;
- edge effects;
- limited data accuracy;
- poor inter-operator and inter-laboratory reproducibility;
- excessive consumables usage;
- high consumption of tips and bulk reagents.

Serial dilutions also typically require a tremendous effort in upfront preparation, either to manually calculate and set up a dilution, or to program automated processes.

Direct titration: a game changer for IC_{50} determinations

Tecan and HP have teamed up to reinvent the drug titration process for small molecules by providing a solution which eliminates the traditional serial dilution process, going from concept to experiment to results much faster and giving access to compound studies that were previously impractical.

Developed by HP and distributed by Tecan, the HP D300 Digital Dispenser has been designed for drug titration studies of small molecules in DMSO. This compact benchtop instrument enables direct titration of 13 pl to 10 µl volumes with single use Dispenseheads, allowing dose-response curves to be created directly from stock compound solutions and completely eliminating the need for serial dilutions.

Entering the picoliter world is a challenge in itself – due to the increasing surface-to-volume ratio and evaporation risks – but HP has led the world in the development of inkjet printing technology, reliably dispensing picoliter droplets around the world for over 20 years. This expertise has been applied to drug discovery, accurately dispensing thousands of droplets per second and bringing speed, reproducibility and standardization to the titration workflow.

In a structure-activity relationship (SAR) study, manual dilution was compared with autopipettor dilution and the HP D300 Digital Dispenser. For the 30 diverse compounds included in the study, direct titration offered an equivalent pIC_{50} to manual and autopipetting. Compared to an autopipettor, a two-fold improvement of the standard deviation (0.08 vs 0.18) and improved performance (Hill coefficients of 0.96 and 0.85 respectively) were achieved.

Figure 1 HP D300 Digital Dispenser dispensing to a 384-well plate

HP T8 Dispensehead cassettes

The HP D300 system makes use of single use disposable cassettes to reduce the risk of contamination. Dispensing itself is also contactless and direct, eliminating serial dilutions and the potential for carry-over or cross-contamination. The instrument can handle both 96- and 384-well SBS-format plates, and compounds from an individual Dispensehead can be titrated into up to five dry or assay-ready plates for biochemical or cell-based studies.

The instrument is very easy to set up and the user-friendly software guides you through the operational steps, allowing you to program and run your first experiments quickly. The software can even calculate the right compound volume, based on the desired concentration, eliminating calculation errors.

Any dose in any well – the choice is yours

The HP D300 allows you to define any plate map, offering non-contact dispensing of any dose to any well for 'assay-ready' plates. A typical manual experiment consists of eight doses in triplicate of each compound, using 24 wells of a plate. Thanks to the improved precision and reproducibility of the HP D300, it is now possible to create a 16 dose curve, increasing the data points in the slope of the curve, decreasing the standard error and revealing the true shape of the curve, while using fewer wells of the bioassay plate. This would not be feasible when setting up a traditional dose-response curve, due to instrument or manual pipetting limitations, but direct dispensing gives you the flexibility to determine the most time- and cost-effective dose-response curve for your application, without compromising data quality.

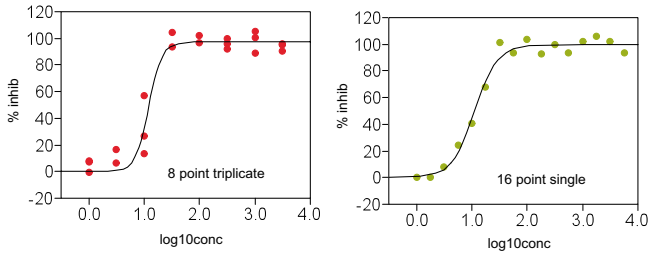

Figure 2 Comparison of an 8 point titration, in triplicate, with a single replicate 16 point titration

Layout	EC_{50}	Standard error
8 point triplicate	12.2	0.049
16 point single	9.8	0.027

Table 1 Comparison of results for 8 point triplicate and 16 point single plate layouts

Randomization to minimize the impact of edge effects

The system's software allows you to randomize samples across the plate to minimize the potential impact of edge effects, ensuring unbiased results. Systematic 'dips' can occur at high/low doses due to edge effects and randomization removes these systematic errors. This ensures a better fit of data to a standard curve, allowing more accurate determination of IC_{50} values.

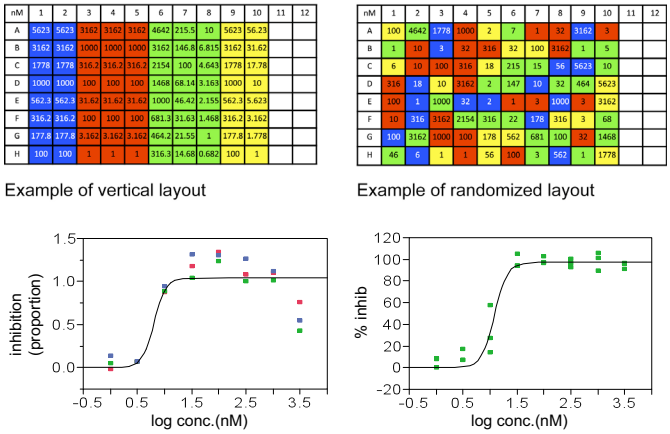

Figure 3 Non-randomized vs randomized assay results (red wells)

High resolution titration

The HP D300's superb flexibility allows you to create high resolution titrations with up to 50 data points/log, eliminating the need for curve fitting and offering excellent active site titration analysis. The 384-point titration shown in Figure 4 demonstrates the system's exceptional working range, allowing direct dosing from ~20 to 100,000 nM with a higher starting concentration and ~0.01 to 20 nM with a lower starting concentration.

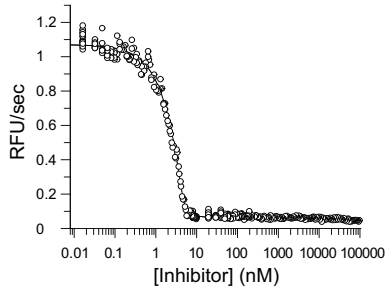

Figure 4 384-point titration dispensed in 6 minutes with the HP D300

Drug interaction studies

Drug modulation studies, such as drug-drug titrations, are of importance for mechanistic studies, oncology and inflammation research – as well as the preliminary study of binding site characteristics – yet are impractical with conventional dilution processes and cross-titration schemes. Using the HP D300 Digital Dispenser, these studies can be easily and accurately performed in a fraction of the time.

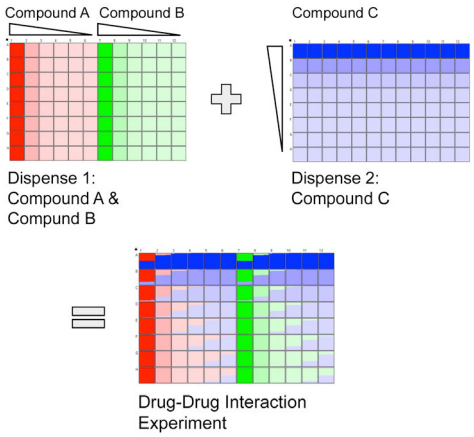

Figure 5 Drug interaction experimental set-up in HP software

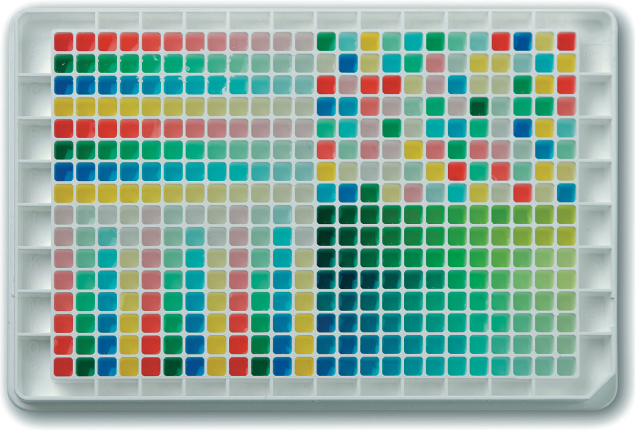

Figure 6 Users can create any plate map desired with the HP 300 Digital Dispenser

Overall benefits

The HP D300 Digital Dispenser offers numerous performance advantages over traditional serial dilutions, as well as previously unachievable applications, including:

- Extended working range – from 13 pl to 10 µl
- DMSO normalization – with backfill functionality
- Reduction of plate edge effects – with randomization function
- Improvement in replicate precision – compared to traditional approaches
- Improved Hill coefficients – through elimination of carry-over
- High resolution dose-response curves – offering high quality compound activity definition
- Up to 50 points/log can be dispensed – making active site titrations possible
- Allows complex drug modulation studies – such as drug-drug titrations
- Easy programming and accurate dispensing in minutes
- Multi-plate dispensing – up to 5 dry or assay-ready plates
- Simplification of dose-response workflows – eliminating the need for intermediate plates
- Intelligent software – for calculation of volumes and concentrations
- Optional shaking of destination plate during dispense

The HP D300 Digital Dispenser's exceptional design and performance has already resulted in several innovation prizes, including:

SLAS 2012 New Product Award Winner

ELRIG Drug Discovery 2011 Technology Prize Winner

Frost & Sullivan 2011 Best Practices Award

HP D300 Awards

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