Digital Dispensing for Direct Dilution: New Flexibility in Dose-Response Analyses

Jeff Nielsen (jeff.nielsen@hp.com), Ken Ward, Christie Dudenhoefer, David Ochs, Joshua Yu, and Kevin

Digital Dispensing Overview:
Digital dispensing enables dose-response analyses without the use of serial dilution. This direct-dilution process generates a dose directly from stock solution by ejecting the appropriate number of picoliter droplets into each well. This means each dose is independent of every other well. Intermediate and serial dilution steps can be eliminated, enabling simplified workflows. Dispensing is accomplished by jetting droplets into either dry or previous filled wells.

In addition to the economic advantages of improving speed, precision, compound usage and waste, this method has scientific advantages. This includes making the execution of complicated experiments easy and fast. For example, direct dilution makes it practical to routinely execute drug-drug interaction (synergy) experiments and to employ finely-spaced doses with each well at a unique dose level.

Method:
The ease and flexibility with which plate dispensing protocols can be created and executed with a non-contact digital-dispensing technology enables certain types of experiments that would not otherwise be practical.

Example 1 - Targeted Titrations:
Digital dispensing does not have a time or cost penalty associated with having every dose level be unique, and thus it is practical to utilize singlicates, rather than replicates to improve curve fits. Furthermore, non-uniformly-spaced singlicates can be easily created using the instrument software (Figure 5), with densely-spaced doses near the IC50 value, and sparsely-spaced doses far from the IC50 value. This enables better utilization of wells by putting doses where they are of most value.

Example 2 – Drug-drug Interaction:
While synergistic/antagonistic experiments are complicated to design and execute with analog dispensing technologies, they are easy with digital technology, even when every well has a unique combination of two or more drugs. The titration dose-levels for two drugs are created independently of one another with a software wizard (Figure 6) and the software subsequently conjoins the dose levels so that every dose level of drug one is combined with every other dose level of drug two. One dispensehead is used for each drug and the two dispense patterns are dispensed sequentially and independently of one another.

Results: Drug-Drug Interaction:
The top portion of Figure 7 shows software screenshots from a typical co-titration, with each color representing a drug, and each level representing a concentration. The total time to program this protocol and dispense this plate is five minutes.

In the left Synergy-surface graphic, drug A is co-titrated against itself to show what a non-synergistic response looks like. The response surface in the graphic is normalized relative to a simple additive response. When a drug is co-titrated against itself, the results are additive and the response surface is flat (blue). In the right Synergy-surface graphing synergic response, drug A is co-titrated against drug B. Here, a synergistic response between the drugs is seen at certain ratios. The response surface is non-intuitive, but real, showing the power of these experiments.

Outstanding Precision:
Key to the performance of the HP D300 Digital Dispenser is excellent precision over a wide dynamic range. Three assay plates were created to assess the instrument precision across five orders of magnitude: one high volume absorbance-based plate and two fluorescence-based plates. Four test volumes were dispensed per plate and ten replicate wells were dispensed per Dispensehead at each volume.

Dispensing precision is shown in Figure 8. For volumes greater than 1nL, the within-head CV is around 2%. When dispensing a single 0.013nL drop, the CVs are slightly above 10%.

Conclusion:
Direct dilution using HP picoliter digital dispensing technology is a replacement for serial dilution. Compound usage can be reduced by 90%, while reliability and speed are improved with simplified workflows. Non-contact dispensing of droplets directly into assay wells make it practical to routinely perform drug-drug interaction experiments.

This non-contact digital dispensing system frees the researcher from the constraints of analog-based dispensing methodologies, enabling complicated experiments that have not been practical with manual or robotic serial dilution methods. Visit Tecan to discover how to bring HP's new picoliter dispensing technology into your laboratory.