Acoustic Droplet Ejection Improves Dose-Response Determinations

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Abstract

Dose-response experiments, such as IC₅₀ analyses, are time- and labor-intensive. They usually require multiple serial dilutions and large amounts of sample. Acoustic liquid displacement (ADE) transfers fluids in discrete droplets of 2.5 nL. Both ADE transfers and direct DMSO transfers are shown. 5 µL of sample is required for a single analyses and 13 µL if run in triplicate. This method also uses 57 µL of DMSO for a single analysis and 90 µL when run in triplicate.

The final amount of DMSO in each assay well by the manual method is 1 µL. To keep the final concentration of DMSO below 1%, the manual method requires an assay volume of 300 µL. Recent publications have shown the potential for significant assay problems when the DMSO concentration is higher. This significantly reduces the potential for assay miniaturization.

Conclusion

ADE transfers fluids in discrete droplets of 2.5 nL. Both source plate and intermediate plate would typically be 384-well format but a 96-well format is shown for simplicity. Earlier high-throughput screening assays would have used 1 µL of each well to have a single compound. Only these compounds would be sampled (“cherry-picked”) in the IC₅₀ experiment. Material would be transferred from the source plate to the assay plate in four different amounts. Then that same source plate would be used to transfer two volumes of material to the intermediate plate. Those volumes would be flled with 25 mL DMSO to obtain 100.1 and 100.001 mL solution. Note that these are not serial dilutions. Fluid from the intermediate plate would be transferred to the assay plate to establish a 12-point, 6-log concentration range.

Acoustic Droplet Ejection (ADE) uses sound to move liquids and eliminates all physical contact with the solution being transferred. There are no pipette tips, no pin tools and no nozzles. Besides reducing costs of consumables, the elimination of physical contact with the solution dramatically improves both precision and accuracy of transfer to best-in-class. Typical transfers of 2.5 nL have CV < 4%.

Because ADE can precisely and accurately transfer nanoliter volumes of DMSO sample directly to assay plates, dilutions can be eliminated or significantly reduced to a single, non-serial step. This eliminates the problem of samples crashing out of solution while maintaining a very low concentration of DMSO in the final assay. The low volumes accessible to ADE transfers also provides a mechanism to minimize assay volumes to 1536-well format – a move that saves significantly on assay reagent costs.


Conclusions

ADE improves IC₅₀ analyses in the following ways:
• Improved results with fewer false negatives. Probably due to elimination of compound precipitation
• Less error, better accuracy per transfer (>5% CV typical)
• Fewer serial transfers – less accumulated error
• Less sample used (save at least 90-95%)
• Less DMSO in final assay (reduction of 97-99%)
• Potential for miniaturization thereby saving assay reagents
• Less DMSO waste is generated (by many liters)
• Significant savings on consumables
• Eliminates manual steps