

Combining low and high volume liquid handling capabilities for ADME screening

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introduction

Current pipetting technologies allow flexible pipetting only down to ~1 µl with sufficient precision and accuracy. In addition, volumes > 0.5% of DMSO, used as a solvent for compound collections, can inhibit enzyme activities.

To allow assays to be performed faster and using less reagents a fast, high quality nanolitre dispensing system is required.

In this study we demonstrate the integration of two liquid handlers to extend the volume dispensing range creating low-volume assay-ready plates with high accuracy and precision. This was then successfully applied to a CYP inhibition assay.

mosquito HTS's ability to miniaturize assays means much smaller volumes, resulting in saving on resources such as buffers, assay kits, radio-ligands, and enzymes.

In addition, this integration could reduce the volume of DMSO waste and decrease the amount of valuable compound used by up to 16-fold.

1. TTP Labtech mosquito® HTS

TTP Labtech's mosquito HTS is a nanoliter liquid handling instrument combining the advantages of a disposable tip system with those of a positive displacement pipette.

Main properties:

- positive displacement tips: 25 nL – 1, 200 nL
- disposable tips: no cross-contamination
- high throughput, multiple dispensing with 8 or 16 tips
- high storage density (36,000 tips on a spool)
- low dead volumes
- low maintenance and better reliability

The mosquito HTS liquid handling system is ideal for:

- low volume assay-ready plate generation (25 – 1,200 nL)
- low volume serial dilutions <1 µL
- reformatting of plates from 96 up to 384/1536



Figure 1. mosquito HTS

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2. Tecan Freedom® EVO

The Tecan Freedom EVO System is a robust and flexible liquid handling platform from 50 – 5,000 µL which allows performance of pipetting, shaking, incubation and plate handling operations.

Many third party devices can be also integrated such as incubators, plate washers and readers.



Figure 2. mosquito HTS integrated with the Freedom EVO system

3. validation

Validation of the mosquito HTS at dry dispensing DMSO into a 384 well microplate demonstrated highly accurate and precise results with %CV of 1% for 250 nL.

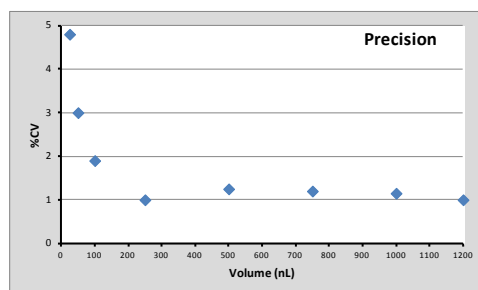
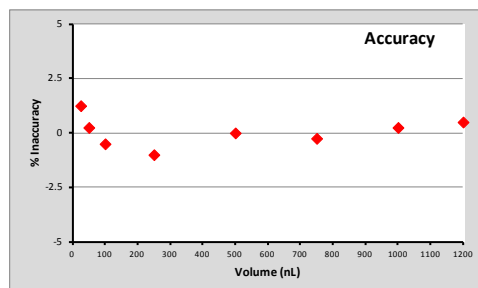


Figure 3. Accuracy and precision for DMSO dry dispensing

4. CYP inhibition assay

mosquito HTS performed a 2.5 fold serial dilution from a DMSO stock solution of ketoconazole (n = 48) using volumes of 1.8 µL of 100% DMSO + 1.2 µL transfer volume.

Transferred to CYP IC₅₀ assay plates (up to 5 assay ready plates with 250 nL can be made from one aspirate of the serial dilution).

The CYP inhibition assay in human liver microsomes was completed using the Tecan Freedom EVO and CYP enzyme activity was measured with a RapidFire system (Agilent Technologies, UK) coupled with a API 5500 mass spectrometer.

4. results



Figure 4. mosquito HTS 25 - 1,200 nL linear dry dispense

Ketoconazole inhibition of CYP inhibitor-mediated midazolam 1'hydroxylation in human liver microsomes produced an IC₅₀ of 0.024 µM.

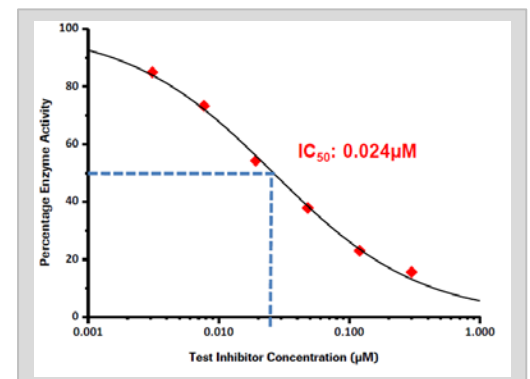


Figure 5. Concentration-dependent curve for a CYP inhibitor

The IC₅₀ of the test compound was calculated and showed excellent test to test comparability consistent with literature values and current assay.

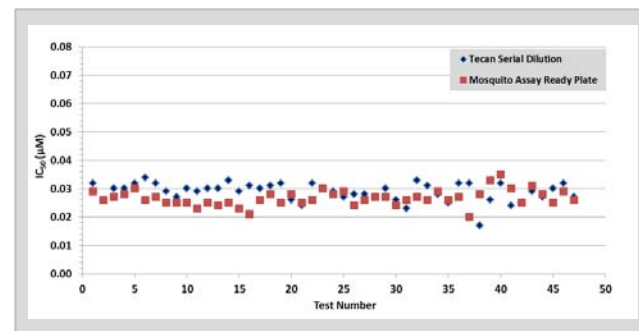


Figure 6. IC₅₀ data consistent with literature values and current assay

conclusion

This is the first time mosquito HTS has been integrated into Tecan platform.

Excellent data quality was obtained from this integration for a CYP inhibition assay.

Future applications that could benefit from this type of integration:

- other assays that require assay ready plates
- generation of standard curves
- high dilution factor preparation activities e.g. MS analysis plates for solubility assays

acknowledgements

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