Rapid Homogenization of Single Step Dilutions in Miniaturized 1,536 MTP Liquid Handling

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keywords: miniaturization, 1536 MTP, low volume liquid handling, nanoliter, pin tool, high speed video imaging, compound mixing, DMSO, cellular assay

INTRODUCTION

One of the major needs in increasing the probability on finding new biomedical entities in Pharmaceutical Industry is – besides others – to

• increase throughput of automated drug screening and
drug testing. This allows higher integration and robot friendliness (clamp modification / lift mechanism)

• save consumption of the limited compound library.

One step into this direction is miniaturization of assay volumes and drug screening. This allows higher integration and robot friendliness (clamp modification / lift mechanism)

Along with this, low volume liquid handling both faces physical effects resulting from its increased surface to volume ratio (surface tension, diffusion, evaporation, viscosity, ...) and also often increases the relative dead volume (= volume needed in assay / total volume taken from compound stock).

The goal from our customers was to optimize the relative volume by still increasing the throughput. The market showed no ready solution to have. So we linked our customer needs with our know-how and the market resources to get what we need.

METHODS & RESULTS

Some years ago Pin Tools were not very well accepted due to their poor accuracy, strongly depending on minor changes in surface tension and viscosity of the solution (e.g. by DMSO or just by temperature), and also on source / destination volume (i.e. dipping height). Since V&P came with slotted and hydrophobic coated pins they were tested on their robustness and reproducibility.

The nature of the DMSO/H2O interface is diffusion hindered and therefore mixing by diffusion is very bad and needed to be forced. A just recently launched high speed shaker prototype by H+P (up to 8'000rpm) was tested by viewing with a high speed imaging device (up to 8'000fps) the process directly inside a well of a 1'536MTP. This viewing process allowed an effective parameter optimization for the mixing process in the well, especially with DMSO, which gave direct input to finalize, in collaboration with H+P, the first high speed shaker for 1'536MTPs on the market.

CONCLUSION & SUMMARY

• Pin Tools, slotted and hydrophobic coated, can and will be used for direct miniaturized compound transfer in uHTS with low relative dead volume.

• Miniaturized volume transfer directly from stock to assay plate is possible within any intermediate dilution steps in an acceptable variance range.

• Miniaturized direct volume transfer by Pin Tools in 1'536MTP wells (or pipetting, in preparation) is most effective by high speed shaking (<300ms in a 1'536well), i.e. usable for cellular assays.

• High Speed Shaker developed in collaboration with leading manufacturer H+P for:
  – 1'536MTPs
  – 384MTPs (increased frequency range)
  – robot friendliness (clamp modification / lift mechanism)

ABSTRACT

Assay miniaturization into 1'536MTP, accompanied with low volume handling, is one important step in pharmaceutical industry in increasing the probability in finding new biologically active marine entities by continuously increased HTS. This volume range leads to minimized physical challenge (surface tension, diffusion, evaporation, viscosity) caused by surface-volume ratio. However, this results in increased relative dead volume to transfer compound solution directly from stock to assay high speed video visualization of the compound transfer and its dissolution revealed nonhomogenous high compound/DMSO concentrations in the assay volume, which could end up in misleading results, especially in cellular assays.

Therefore, the launched high frequency shaker prototype has been increased in collaboration with a leading manufacturer for its mixing efficiency and its use in fully automated systems, resulting in homogeneous distribution of compound/DMSO in a 1'536MTP well in fractions of a second.

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