Low Volume Liquid Handling of Organic Solvents for Compound Storage and Dissolution using mosquito®

Gillian Lewis, Tristan Cope, Joby Jenkins, David Gledhill & Rob Lewis
TTP LabTech Ltd, Melbourn Science Park, Melbourn, Hertfordshire, SG8 6EE, UK

Abstract

The technology of storing dried microquots is an interesting new idea which aims to reduce very significantly the amount of material – and in particular of intermediate material – wasted in the process of synthesising new compounds. This technology may also benefit drug screening where there is a similar need to conserve material and where there are also concerns of material stability.

mosquito® (TTP LabTech) is a positive displacement liquid handling system capable of pipetting solutions with a high degree of accuracy (to within 5%) and precision (CVs of <10%) in the nanolitre range.

Here, we demonstrate mosquito's ability to dispense accurately low volumes of organic solvents of low surface tension and to re-dissolve compounds which have been previously dried in storage wells.

Conclusion

mosquito® is capable of:

- performing an initial solution dispense
- adding the solvent to re-dissolve dried compound at the required concentration.
- mixing and transferring the required volume to another location.

This work also resulted in a TTP LabTech designed plate optimised for compound storage. Solid recovery results obtained using other combinations of conventional SBS plates and organic solvents proved unworkable or unsatisfactory.

This work demonstrates the basic feasibility of this technique to prepare a dry storage material which remains stable and can be later reconstituted to produce low microlitre volumes across a range of molarities.

This procedure can be applied to a useful range of compounds and standard solvents, in particular low surface tension solvents that could not be accurately pipetted using air displacement pipetting systems.

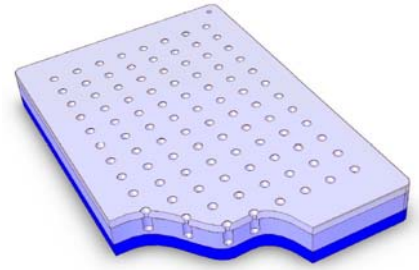
1 Plate Design

Various SBS-format plates were tested as storage systems. The requirements for suitability were:

- compatibility with the majority of organic solvents
- a conical well bottom to hold the solvent droplet in a confined space (necessary for solvents with low surface tension).
- a well diameter that is as low as practically possible, in order to restrict dead volume, but not so low that solvent wicks up between pipette tip and well wall.
- a suitable lid to prevent evaporation. Seals or lids that can be pierced by mosquito pipette tips were found to have adhesive which dissolved when exposed to solvent vapour during the time taken for re-dissolution.

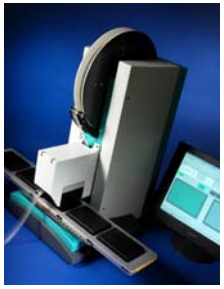
Unfortunately, no SBS-format plates were found that fitted all of these criteria.

To address this, TTP LabTech designed and made a plate from PTFE to be compatible with most chemicals and solvents. The wells were cylindrical and had a conical base. In order to minimise solvent loss, the wells were designed to have a very low volume and thus less dead space for solvent to evaporate into. The smallest practical diameter well was used and depth optimised to reduce the dead space volume available for evaporation. The lid was made from metal with a hole diameter slightly larger than the mosquito tip diameter of 0.8mm.



The TTP LabTech storage plate in 96 well format

2 mosquito® Instrument



mosquito is a low volume liquid handling instrument combining a low-cost disposable tip system with a positive displacement pipette to ensure zero cross-contamination.

mosquito is capable of pipetting and mixing volumes from 1.2µL down to 50nL with no washing required.

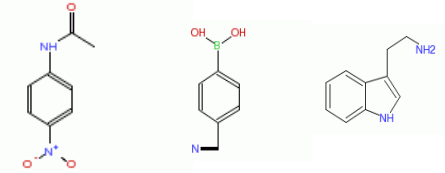
3 Test Procedure

- A concentrated solution of each test compound was pipetted into a glass source microplate (96 well) and a foil lid placed on top.
- mosquito was used to pierce the foil seal and pipette the concentrated solution into the storage plate and the solvent allowed to evaporate.
- The solvent required to re-dissolve the compound was then pipetted into a glass plate and mosquito was used to add solvent to the compound dried in the wells.
- After a 1 minute pause, mosquito pipette tips were used for a 10 x 1000nL mix. Material for analysis was removed from the storage plate using mosquito.
- The material removed was placed in an analytical quartz plate for absorbance measurement. Each compound was read against 10 standards and percentage recovery with 95% confidence interval and % CV calculated.

mosquito settings were optimised to ensure the drops were located in the centre of the well and to minimise drag by the tips up the sides of the well. In particular, we slowed down aspirate and dispense speeds and tip clear speeds (this is the speed at which the tip pulls away from the base of the plate).

4 Compound Recovery Performance

Three compounds were selected for extensive profiling based upon their ease of detection in the presence of organic solvents.



1 2 3

4-nitroacetanilide | 4-cyanophenyl boronic acid | tryptamine

Cmpd	Solvent for initial dispense (volume)	Solvent for re-dissolution (volume)	Percentage of required value (+/- 95% CI)	% CV
1	THF (1000nL)	THF (3800nL)	**108** +/- 1.91	3.33
1	THF (2000nL)	THF (3800nL)	**108** +/- 1.43	2.50
1	THF (3000nL)	DMSO (3800nL)	**109** +/- 2.34	1.36
1	DMSO (400nL)	THF (3600nL)	**94.1** +/- 1.78	3.55
2	MeOH (600nL)	MeOH (6000nL)	**104** +/- 2.93	5.28
2	MeOH (1800nL)	MeOH (6000nL)	**104** +/- 0.578	1.04
3	MeOH (1000nL)	MeOH (4000nL)	**106** +/- 2.32	4.11
3	MeOH (1500nL)	MeOH (4000nL)	**108** +/- 1.76	3.07
3	MeOH (2000nL)	MeOH (4000nL)	**108** +/- 2.11	3.66
3	MeCN (2000nL)	MeCN (4000nL)	**110** +/- 4.96	8.49
3	MeCN (400nL)	DCM (4000nL)	**106** +/- 4.40	7.80
3	DCM (2000nL)	MeCN (4000nL)	**108** +/- 8.33	14.5
3	DCM (400nL)	MeOH (4000nL)	**108** +/- 10.0	12.3
3	DCM (1000nL)	MeOH (4000nL)	**101** +/- 5.28	9.84
3	DCM (2000nL)	MeOH (4000nL)	**102** +/- 9.07	15.8

Number of replicates = 16 for each compound/solvent dispense volume and re-dissolution volume combination.

Where greater than 100% recoveries are found the assumption has been made that the entire compound has gone into solution and the concentration increase above 100% is due to reduction in solvent volume caused by evaporation.