High Frequency Focused Acoustic Technology: Evaluation for Compound Mixing and Dissolution

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Compound Management and high throughput screening can both be impacted by incomplete compound dissolution as well as precipitation, the latter occurring on storage or after addition of aqueous buffer. To date, mixing and dissolution have been performed using vortexing, sonication or centrifugation. However, all these methods have drawbacks.

In addition membrane preps are prone to aggregation and can be highly viscous making them difficult or impossible to aspirate and dispense accurately.

In this poster we present our efforts at GSK to evaluate Adaptive Focused Acoustics "AFA", in ultra High Throughput Screening (uHTS), Compound Management (CM) and the homogenisation of Membrane preps (uHTS).

Technology

Adaptive Focused Acoustics has evolved from the highly developed Lithotripsy Kidney stone treatment and ultrasound imaging industries. AFA is a patented technology from Covaris Inc. It works by sending high frequency acoustic waves from a dish-shaped transducer. These converge to a small-grain of rice sized localised area (figure 1) creating intense mixing.

The Covaris acoustic transducer operates at 500khz with a wavelength of ~1mm, unlike conventional sonics which has a wavelength of ~100mm.

This enables the acoustics energy to be exactly directed into 4ml vials to 1536 plates in a non-contact and isothermal mode (figure 2).

Introduction

fig 1

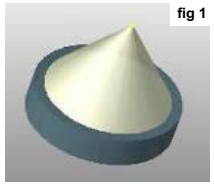
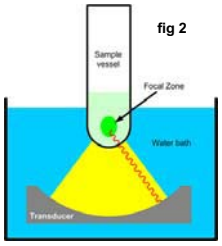


fig 2

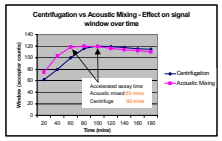


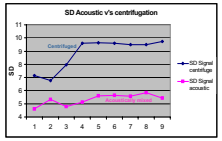
Acoustic Mixing Applications

Ultra High Throughput Screening

Currently, mixing of fluids in 384 and 1536 plates is not controlled. We have performed an evaluation of AFA novel technology which can actively mix small fluid volumes and consistently improve performance of several parameters.

uHTS Data

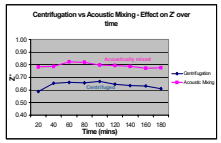




HTRF cAMP Detection Assay

The CisBio HTRF cAMP detection kit is currently on trial at GSK.

This experiment investigated agonist performance in a G protein coupled receptor assay.



The experiment was carried out in 1536 white Greiner plates with 50nl/well of compound with a total assay volume of 8ul.

Assay protocol:
4 x 2ul reagent additions using Synquad and Cybiwell
Centrifugation step after each addition
Final centrifugation step was replaced with acoustic mixing.
Acoustic mixing was achieved using an 800kHz Covaris line based acoustic transducer (figure 3) which allows a single pass down a 1536 microtitre plate in order to mix.
This instrument was a prototype and a full automation user requirement specification is being created to determine specifications and impact within the uHTS environment (figure 4).

fig 3

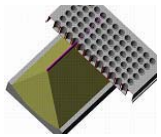
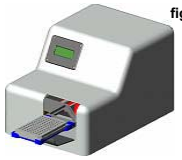


fig 4



uHTS Results & Conclusion

The data shows an overall improvement in assay performance in terms of Z'. This is particularly apparent in the case of the cAMP HTRF assay, where Z' values are improved by a factor of **0.2**

Additional benefits, particularly in the HTS scenario, are accelerated assay time frames, non-contact mixing, and ease of automation.

The time it takes for the assay to reach equilibrium is also reduced by **30%** (and hence maximal signal is attained earlier). The reduction in both mixing time and time to reach maximal signal could increase plate throughput.

Data generated also indicated the positive effect of acoustic mixing on compound solubility. SPA bead based assays should also benefit due to the increased collision frequency of the binding partners at the receptor-liquid-bead interface. Current assay performance can be compromised due to bead settling and inefficient static diffusion; resulting in long incubation times.

These results are from preliminary experiments. Nevertheless, all results appear to positively impact both the quality and throughput of the assays tested. To define the technical limits on assay performance and throughput for uHTS, a more detailed investigation is in progress.

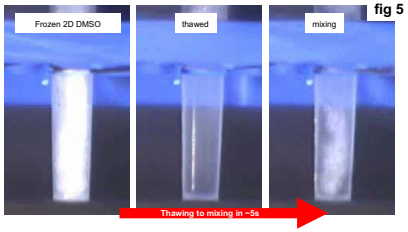
Compound Management

Rapid and effective dissolution of compounds is a primary requirement in Compound Management. To date, mixing and dissolution have been performed using vortexing, sonication or centrifugation. Thawing has been achieved with forced air heating.

Acoustic mixing/thawing using the Covaris technology is currently being evaluated for primary and secondary compound dissolution. Our objective is to acoustically thaw, control primary dissolution and have an effective method to re-solublise any compound drop out throughout the whole compound lifecycle.

Figure 5 shows 2D bar-coded tube thawing & mixing on the Covaris E-200. The entire process is carried out in ~5s.

fig 5



Compound Management Conclusions

- No compound degradation (LC-MS) has been observed after 90s at full power.
- Typical treatment time <20seconds
- C2000 platform co-developed (figure 6).
- Homogeneous solutions and suspensions produced.
- Effective for gums, glasses, powders and crystals.
- Capable of rapid thaw/mix on 2D tubes

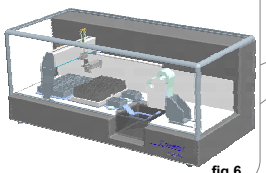


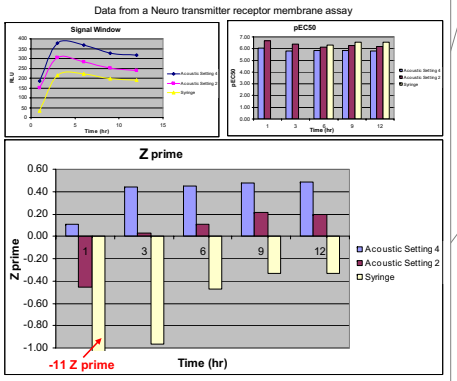
fig 6

uHTS Membrane Prep

Membrane assays are often highly viscous making it very difficult to aspirate and dispense resulting in a poor assay Z'. This high viscosity is thought to be caused by a combination of protein aggregation and high molecular weight genomic DNA.

Current methodology utilises a pre-treatment with a syringe and fine gauge needle to homogenise / shear the membrane preparation prior to liquid handling. In order to improve upon this we have evaluated the Covaris S2 acoustic system for this pre-treatment. We have found that it rapidly reduces this viscosity, improving the preparation homogeneity resulting in improved assay quality (Z').

Data from a Neuro transmitter receptor membrane assay



Treatment conditions: dc = duty cycle, cpb = cycles per burst.
Acoustic setting 2 = 20% dc, intensity 2, 100 cpb, 10s
Acoustic setting 4 = 20% dc, intensity 4 , 100 cpb, 10s
Acoustic treatment was carried out in a 2ml Eppendorf tube at 8°C.

Data: Signal to noise increase ~100%
EC50 assay equilibrium is attained after 1 hour (6 fold decrease.)
Increased Z prime.

Conclusion: membrane preps can be successfully treated with the Covaris AFA technology resulting in a significant improvement in homogeneity and improved Z prime. In extreme cases high viscosity membranes make liquid handling and assay impossible, by using the Covaris acoustics it is possible to recover and run the assay to completion. It is also possible to carry out and simplify the initial membrane prep itself.

Acoustic Mixing Conclusions

This poster demonstrates that the Adaptive Focused Acoustics Technology from Covaris has a wide range of applications in ultra High Throughout Screening, Compound Management and many other areas throughout GSK

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