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

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Introduction

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 this poster we present our efforts at GSK to evaluate Adaptive Focused Acoustics in ultra high throughput screening (µHTS), Compound Management (CM) and High Throughput Chemistry (HTC).

Technology

Adaptive Focused Acoustics has evolved from the highly developed Lithotripsy Kidney stone treatment and ultrasound imaging industries. Adaptive Focused Acoustics 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-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).

fig 1

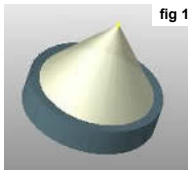
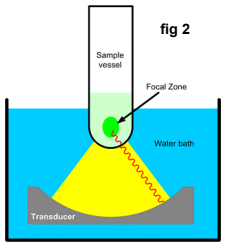


fig 2

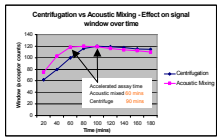


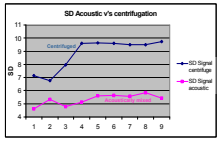
Acoustic Mixing Applications

Ultra High Throughput Screening

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

Preliminary uHTS Data

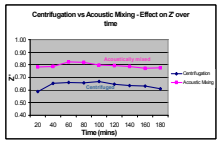




HTRF cAMP Detection Assay

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

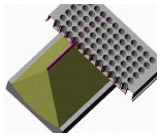
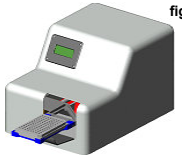


fig 4



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 HTS, more investigation is required.

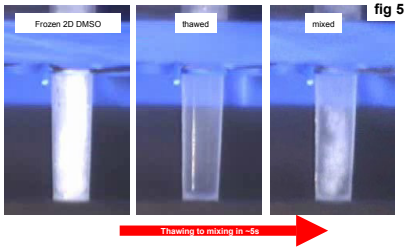
Compound Management

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
- User requirement specification completed for fully automated platform (figure 6).
- Homogeneous solutions and suspensions produced.
- Effective for gums, glasses, powders and crystals.
- Capable of rapid thaw/mix on 2D tubes

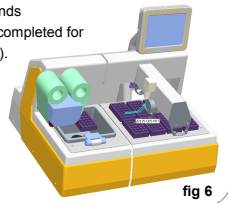


fig 6

High Throughput Chemistry (HTC)

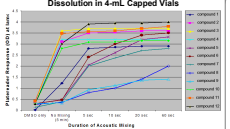
Sample dissolution is a key post-synthesis step requiring 1-3 dissolutions per registered sample in 4 mL vials, HPLC fractions, CleVap collection plates and MTPs.

Current dissolution methods using sonication, shakers and vortexers are still largely a manual, tedious process, resulting in variable results.

HTC's objective is to create definable, standardised processes useful for vials, tubes and Irori CLEVAP plates with better handling for poorly soluble samples.

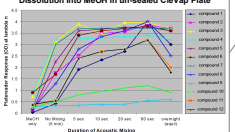
12 compounds were chosen, diverse in structure – basic, acid, non-polar. Included "problematic" samples (poor & slow dissolvers, gums etc).

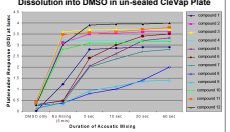
DMSO and Methanol were used as the diluents, various acoustic treatments were applied and optical density (OD) data plotted. The data is shown in Figure 7.



Conclusion

- Efficient mixing in multiple formats has been achieved in sealed 4mL vials and un-sealed CleVap plates.
- Treatment duration > 60 sec, typically < 20 sec.
- Effective for gums, glasses, powders, crystals.
- No decomposition of compounds observed.
- Homogenous solutions and suspensions produced.
- High reproducibility.





Acoustic mixing may become "best practice" for all dissolutions (post-synthesis and building blocks etc.)

Acoustic Mixing Conclusions

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

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