Solubility Rules OK! What ultrasonic mixing can do for you

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Introduction

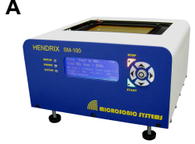
A lack of compound solubility is a major hurdle in High Throughput Screening (HTS) and Lead Profiling as compounds are not always readily soluble in DMSO or remain soluble during storage. These solubility problems can at best result in unacceptable variability in assay reproducibility and at worst result in the apparent inactivity of the compound in the assay.

Additionally, it is important to ensure adequate mixing of compounds and assay reagents in low volume assays performed in small volume 384 and 1536 well plates.

We have evaluated the mixing of compounds and assay reagents in 384 and 1536 well plates using the Hendrix™ SM100 ultrasonic microplate fluid processor from Microsonic Systems. The objectives were two fold: To determine if compound solubility in storage plates is improved following ultrasonic solubilisation. To evaluate if data fidelity in biochemical and cell-based assays is improved when ultrasonic mixing reagents in assay plates.

Hendrix SM100 ultrasonic plate mixing device (**A**) and under bench remote unit (**B**)

A



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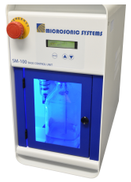
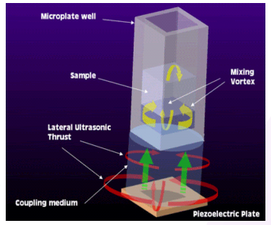


This LUT™ technology is derived from Micro-Electrical-Mechanical Systems (MEMS)-based transducers, which generate ultrasonic waves when excited with RF power. An array of 384 of these transducer-elements were used in the Hendrix to enable high-speed parallel processing of microplates

Well plates were ultrasonically mixed and solubilized using the novel Lateral Ultrasonic Thrust (LUT) TM technology of the Hendrix SM100 mixer.



Physical Chemistry

Fig. 1. Empirical determination of solubility.

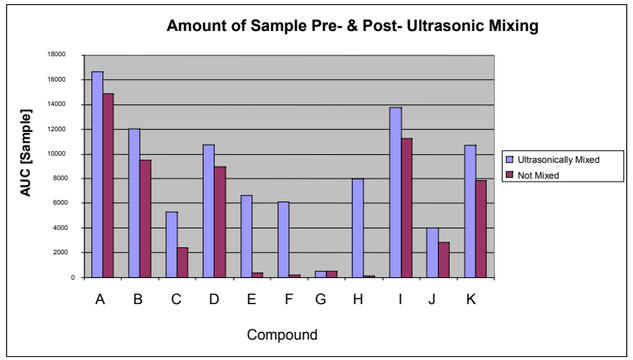


Fig. 1. Almost all compounds were more soluble following ultrasonic mixing. In some cases up to 80% more compound was solubilised as determined by Area Under the Curve (AUC). There were no alterations in Molecular Weight (not shown) suggesting that sample integrity was maintained. Compound A was used as a negative control as this compound is readily soluble in DMSO.

Method: Compounds known to have solubility problems were re-suspended at 5mM in DMSO. Replica plates were generated and either ultrasonically mixed or untreated. Aliquots of compounds were diluted in Phosphate Buffered Saline containing 20mM HEPES (assay buffer) to 10µM and again ultrasonically mixed or untreated. Samples were evaluated in UV-LCMS for molecular weight and AUC as a measure of concentration.

Biochemical Assays

Fig. 2. Comparison of dose-response curves for three poorly soluble compounds screened in a Quenched FRET enzyme assay without (A) and with (B) ultrasonic mixing.

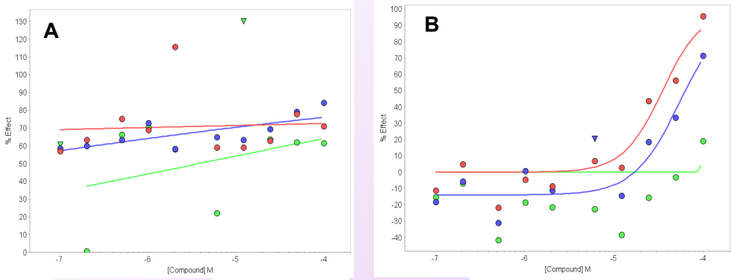


Fig. 2.A. Compounds not ultrasonically mixed did not produce an IC_{50} and showed artefact noise within the assay – baseline is around 65% effect. However, after ultrasonic mixing (Fig. 2.B), two compounds gave an IC_{50} (blue/red circles) and assay noise was removed. We suggest this artefact, which occurs at all compound concentrations, is due to particulate in the well that affects the measuring technology and is removed by mixing.

Method: Compounds were diluted in DMSO to 10mM and dispensed into duplicate 384 well Echo qualified plates (Labcyte). One plate was mixed with the HENDRIX SM100 and the other untreated. The Echo 555 was used to dispense compounds into the enzyme assay plate to generate a 10 point half log dilution series starting at 100 µM final concentration. Two assay plates were prepared from ultrasonically mixed compounds and two assay plates prepared from non-mixed compounds. Compounds that were previously mixed were also mixed within the assay plate with reagents and the assay run to completion.

Fig. 3 Comparison of compound activity before and after ultrasonic mixing of both the compound and assay plates in a biochemical protease assay.

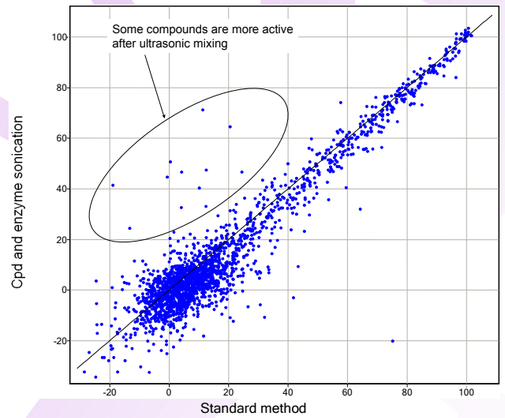


Fig. 3. There was general agreement in the activity of compounds in the assay in the presence or absence of mixing, however some compounds (circled) showed greater activity when ultrasonically mixed. Ultrasonic mixing of the assay components did not affect the activity of the enzyme.

Method: Six 384 well compound plates were assayed in a biochemical protease screen: 10 nL of compound were dispensed from each stock compound plate into 5µl of enzyme (assay plate) using an Echo 555 dispenser. Compound plates were then ultrasonically mixed using the HENDRIX SM100 and 10nL transferred into a second set of assay plates. The assay was initiated by the addition of 5 µl of substrate and one set of assay plates was ultrasonically mixed. All plates were incubated at room temperature for one hour and read on a Perkin Elmer Envision reader.

Cell Assay

Table. 1. Effect of ultrasonic mixing on primary human neutrophils screened within a 7-Trans-Membrane (7-TM) receptor calcium mobilisation assay in FLIPR^{TETRA}.

Table 1. Different ultrasonic mixing conditions have little or no effect on neutrophil responsiveness or ligand potency.

Donor number	pEC_{50} No mixing	pEC_{50} Under "Low" power	pEC_{50} Under "Med" power	pEC_{50} Under "High" power
Donor 1	8.63 8.74	8.55 8.67	8.76 8.93	8.97 8.73
Donor 2	9.12 8.98	8.89 9.11	8.95 9.05	9.13 8.87

Method for cell assay:

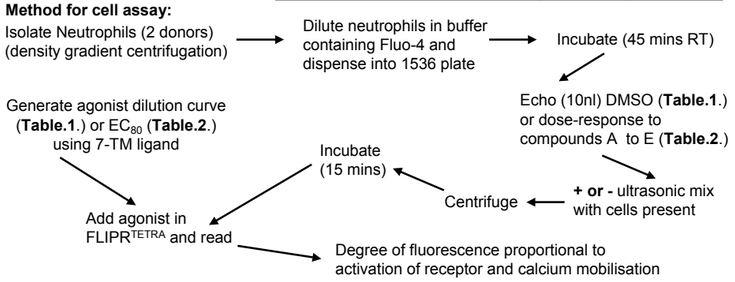


Table. 2. Effect of ultrasonic mixing on the potency of antagonist compounds screened in the primary human neutrophil calcium mobilisation assay above.

Table. 2. Ultrasonic mixing appeared to improve the potency of two antagonists in this assay by as much as one log unit. This suggests that some of these compounds become more soluble when ultrasonically mixed before and during the assay.

Compound	Appearance	Historical pIC_{50} (average)	pIC_{50} No mixing	pIC_{50} Ultrasonic mixing
A	Yellow - clear	6.07	5.95	6.27
B	Colourless - cloudy	6.34	6.23	7.06
C	Yellow - clear	6.21	6.07	6.73
D	Colourless - clear	5.49	5.38	5.52
E	Colourless - clear	5.53	5.49	5.54

Summary

1. Ultrasonic mixing improved compound solubility and appears not to degrade sample.
2. Ultrasonic mixing improved assay reproducibility in biochemical assays probably by enhancing mixing conditions.
3. Ultrasonic mixing does not appear to be detrimental to assay biology or human primary cells.
4. Ultrasonic mixing increased the number of compounds identified as actives in primary screening and the potency of compounds in Lead Profiling.

Conclusions

Ultrasonic mixing will be implemented in Compound Management and HTS during both the plate replication process and to enhance the mixing of reagents in wells and improve assay reproducibility.

Based on its small physical foot-print, the HENDRIX SM100 instrument will be integrated to our current Compound Management and HTS robotic systems.