The Use of Covaris Adaptive Focused Acoustics in PsychiatryCEDD DMPK

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Introduction

In PsychiatryCEDD DMPK sonication and homogenisation is routinely used to aid compound dissolution and tissue sample preparation. Introduction of the Covaris using Adaptive Focused Acoustics (AFA) has enabled the formation of more consistent formulated doses for studies and fine tissue preparations for analysis. Compared to mechanical homogenisation the Covaris is a silent compact instrument which avoids contamination caused by interference of external probes.

Technology

- The Instrument uses AFA which is a patented technology from Covaris Inc. It has evolved from the highly developed Lithotripsy Kidney stone treatment and ultrasound imaging industries.
- AFA works by sending high frequency acoustic energy waves from a dish-shaped transducer (Figure 1). These converge to a small-localised area creating intense mixing. (Figure 2).The Covaris acoustic transducer operates at 500khz with a wavelength of ~1mm, unlike conventional sonics which have a wavelength of ~100mm. This enables the acoustics energy to be focused on samples in glass vials or tubes in a non-contact and isothermal mode, avoiding contamination and degradation (Figure 3). A precise and reproducible control is obtainable with the Covaris.

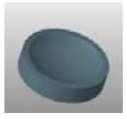


Figure 1

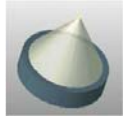


Figure 2

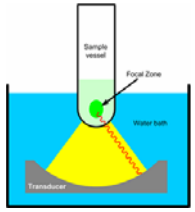


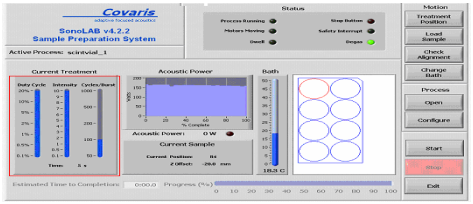
Figure 3

Software

The software, SonoLAB runs the Covaris AFA.
Treatment Settings with different variables can be saved.

Acoustic Treatment Setting Variables

1. Duty Cycle - % on time
2. mV or Intensity – Amplitude of the wave form
3. Cycles / Burst – No of waves applied during the on time



Preparation of Oral doses

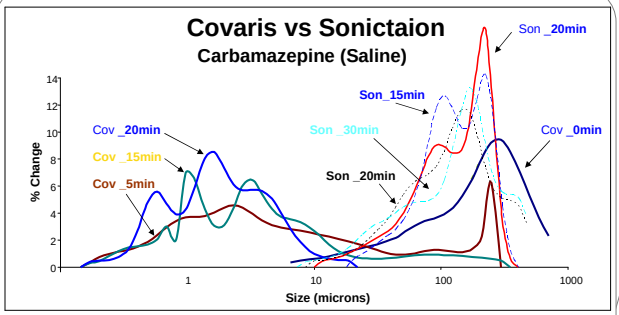
PsychiatryCEDD DMPK studies use oral doses prepared as suspensions in 1% (w/v) methylcellulose *aq*. Previously these have been prepared using magnetic stirring or mechanical homogenisation and sonication. These techniques were mainly, lengthy and not reproducible.
To investigate the utility of AFA for dose preparation we used Carbamazepine, a reasonably insoluble compound was used to compare the Covaris to other preparation techniques.

Method

- 15mL doses of Carbamazepine at 1.5mg/mL in 20mL scintillation vials were prepared.
- The doses were formulated using the optimum conditions for the Covaris at 5, 15, 20 and 30min.
  Duty Cycle – 20% **Intensity – 10** **Cycles / Burst – 500**
- For comparison, Carbamazepine doses were also formulated using sonication and a magnetic stirrer (data not shown) at 15, 20 and 30min.
- The particle size of each dose pre and post formulation was measured using the "Particle Size Analyser_Lecotrac 7.01 LT100"

Results

From the graph shown, it is clearly evident that the Covaris produced a superior po dose suspension compared to sonication. The data shows that the optimum time for minimising particle size to 1um is 15mins (sonication 300um after 15mins), after which the particles begin to increase in size again.



Preparation of Brain and Liver Analysis

For the analysis of compounds in tissue a suitable homogenate is required.

Method

- The tissue is weighed and 2.15mL 50:50 MeOH :H2O per gram of tissue is added.
- Tissues are homogenised using the saved treatment settings in Covaris.
- Compounds are extracted from the homogenate using protein precipitation and analysed by UPLC-MS/MS.

Summary of Covaris Treatment Settings

Duty Cycle	Intensity	Cycles/Burst	Time (sec)
Rat Brain_20mL Scintillation Vial_8 Rack 1.5min			
20%	10.0	100	10
20%	10.0	1000	50
20%	10.0	100	5
20%	10.0	1000	25
Mouse Brain_4mL Vial_8 Rack 1.25min			
20%	10.0	100	30
20%	10.0	1000	45
Rat Liver_20mL Scintillation Vial_8 Rack 3.0min			
20%	10.0	100	60
20%	10.0	1000	60
20%	10.0	100	30
20%	10.0	1000	30

Duty Cycle	Intensity	Cycles/Burst	Time (sec)
Dose (1% Methylcellulose)_20mL Scintillation Vial_8 Rack			
20%	10.0	100	60
DMSO Stock_4mL Vial_24 Rack _Vertical Sweep			
20%	10.0	100	60

Conclusions

The advantages of Covaris over exiting techniques are that it can be used to prepare reproducible, high dissolution oral doses, DMSO stock solutions and consistent tissue homogenates. Overall the Covaris is a versatile, isothermal, silent, closed vessel with no contamination.

References

www.covarisinc.com