New Technology in Tissue Homogenization: Using Focused Acoustic Energy to Improve Extraction Efficiency of Drug Compounds Prior to LC/MS/MS Analysis

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52nd ASMS Conference on Mass Spectrometry
May 26, 2004
Poster: *WPJ 143*

Overview

- Evaluate Focused Acoustics technology for tissue homogenization and compare with Rotary Blade (Polytron) homogenization
- Measure extraction efficiency of drug compounds from homogenized tissue using each process
- Use of Focused Acoustics for tissue homogenization resulted in improved extraction efficiency of drug compounds from tissue samples compared with Polytron homogenization, along with a simplified workflow and automated sample processing

Introduction

Current technology used for tissue homogenization prior to LC/MS/MS analysis primarily uses rotary blade (Polytron) homogenization devices, presenting challenges in throughput and efficiency, including: requirement of cleaning the blades between samples; potential for carryover; and difficulties in processing certain types of tissue. To overcome some of these challenges an alternative approach, using Focused Acoustics energy for tissue homogenization, was evaluated to characterize extraction and recovery of drug compounds from tissue samples prior to LC/MS/MS analysis. This new device offers an alternative approach providing improvements in tissue homogenization, including: automated robotic sample handling; increased extraction levels and reproducibility for drug compounds; "non-contact" sample processing – no cleaning required or potential for carryover between samples; and the ability to efficiently process difficult tissue types like heart and muscle.

Methods

The Focused Acoustics tissue homogenization process and system are described in *Figures 1, 2, 3 and 4*.

Recovery of ^{14}C-labeled Drug Compounds from Tissue:

Animals (Rat) were dosed with ^{14}C-labeled *Drug Compound A*; Heart or Muscle tissue sections (200 mg to 400 mg) were deposited in sample tubes containing 1 mL of water and then subjected to either Focused Acoustics energy (*Covaris E200*; Covaris, Woburn, MA; shown in *Figure 1*) or Polytron homogenization; total processing time was 3 min. per sample; resulting homogenates were protein precipitated and ^{14}C activity was determined separately for supernatant and pellet using scintillation counting.

Recovery of a different, non-labeled, drug compound determined by LC/MS/MS quantitative analysis:

Animals (Rat) were dosed with *Drug Compound B*; Heart and Muscle tissue sections (150 mg to 200 mg) were homogenized using the same methods listed above; samples were protein precipitated and analyzed using LC/MS/MS to quantitate recovery of drug compound.

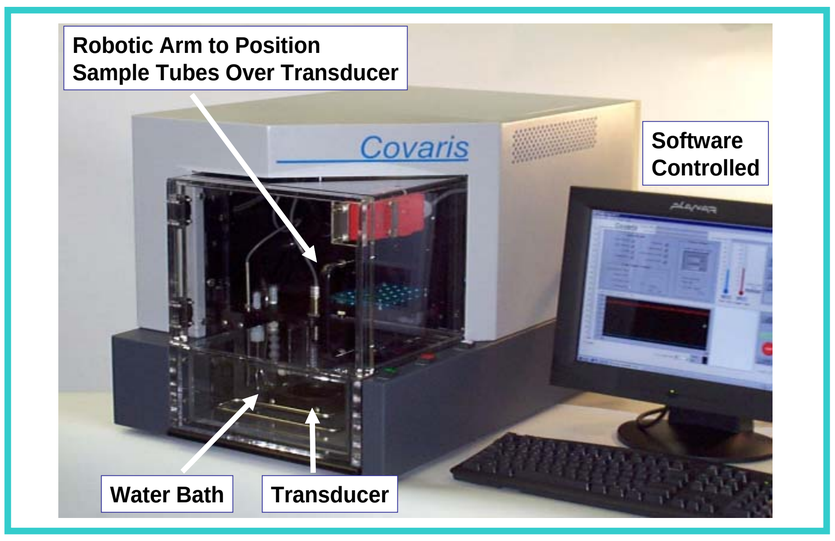

Figure 1. Picture of the *Covaris E200* Focused Acoustics system. Sample tubes containing tissue sections in solution are positioned in water bath over Transducer one at a time for automated processing of up to 24 sample tubes in a single run

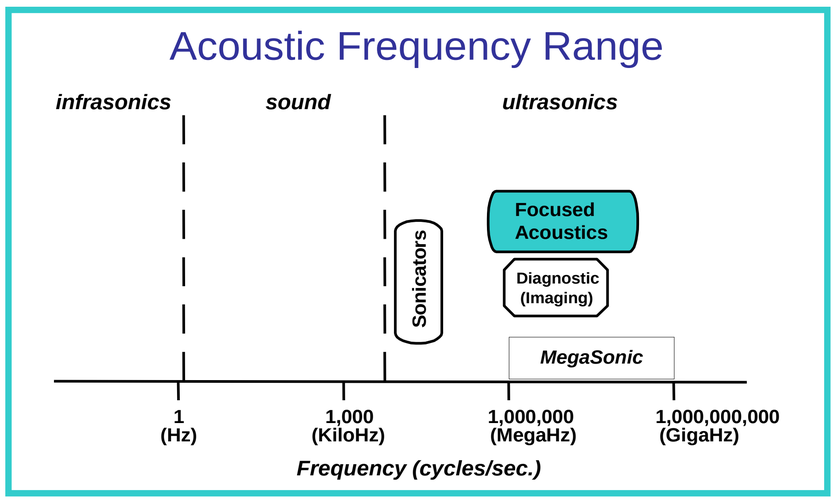

Figure 2. Diagram displaying Acoustic Frequencies; *Sonicators* are shown in the KiloHz region and *Focused Acoustics* in the MegaHz region

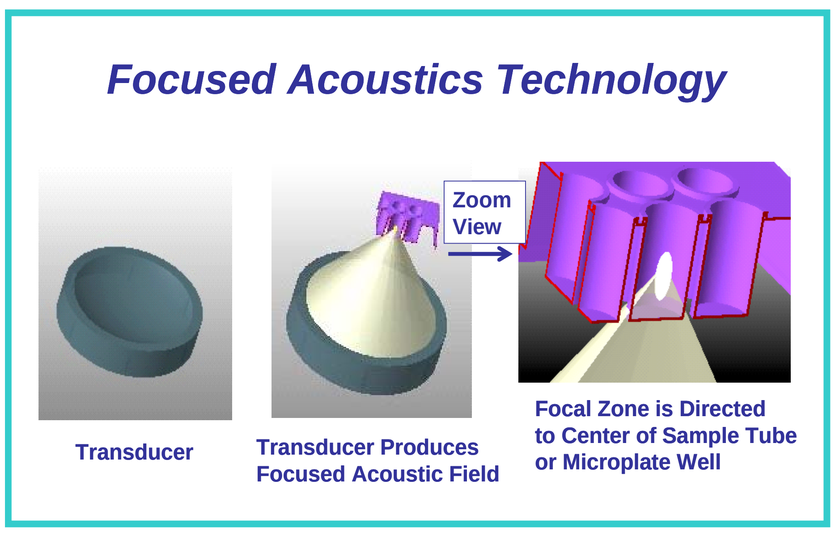

Figure 3. Diagrams showing "dish-shaped" Transducer producing Focused Acoustics Field creating a focal point at the top of acoustic field "cone" and directed inside Sample Tube or Microplate Well; this process produces energy for tissue homogenization

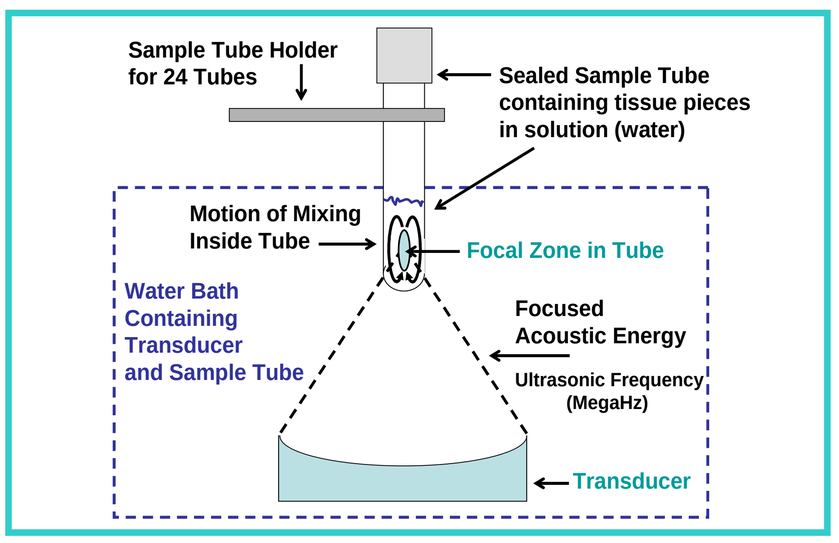

Figure 4. Diagram showing "cross section" of the Focused Acoustics tissue homogenization process; the *Focal Zone* located in the *Sample Tube* delivers the energy for tissue homogenization while simultaneously mixing the sample during the process

Results

Recovery of a ^{14}C-labeled drug compound from tissue was studied to determine extraction levels and amount of drug still remaining in tissue homogenate pellet following homogenization and protein precipitation; recovery levels observed using Focused Acoustics homogenization, shown in *Figure 5*, averaged 94.2% (0.9% RSD) for *Drug Compound A* extracted from Heart tissue; in contrast to this, the recovery using Polytron homogenization averaged 71.7% (17.0% RSD) for the same drug compound, displaying significant improvements in both extraction efficiency and reproducibility using Focused Acoustics homogenization. The recoveries of *Drug Compound A* from Muscle tissue were similar using either Focused Acoustics (93.6%, 0.8% RSD) or Polytron (93.2%, 0.6% RSD); this may indicate that for this compound Muscle tissue was not as problematic as Heart tissue for Polytron Homogenization.

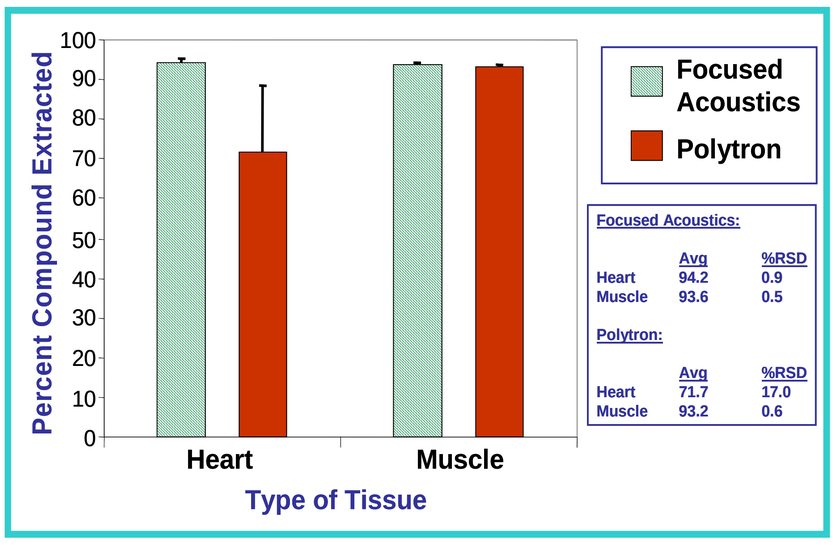

Figure 5. Chart displaying total percent ^{14}C-labeled *Drug Compound A* extracted from rat heart *(n=4)* or muscle *(n=3)* tissue, measuring total ^{14}C isotope activity, comparing results using either Focused Acoustics or Polytron; actual numbers for %Avg. and %RSD are shown in table inset

Results *(continued)...*

Quantitative results for *Drug Compound B* recovery from tissue samples determined by LC/MS/MS analysis, shown in *Figure 6*, displayed an increase of 1.7 times the extraction level from Heart tissue using Focused Acoustics homogenization compared with results using Polytron homogenization. An even larger increase in extraction efficiency was observed for Muscle tissue, resulting in 3.8 times the extraction level using Focused Acoustics homogenization compared with Polytron homogenization. In contrast to this, the extraction levels for Brain and Liver tissue were similar using either Focused Acoustics or Polytron homogenization; this may indicate that the "softer" tissue types may display less differences due to thorough homogenization using either process.

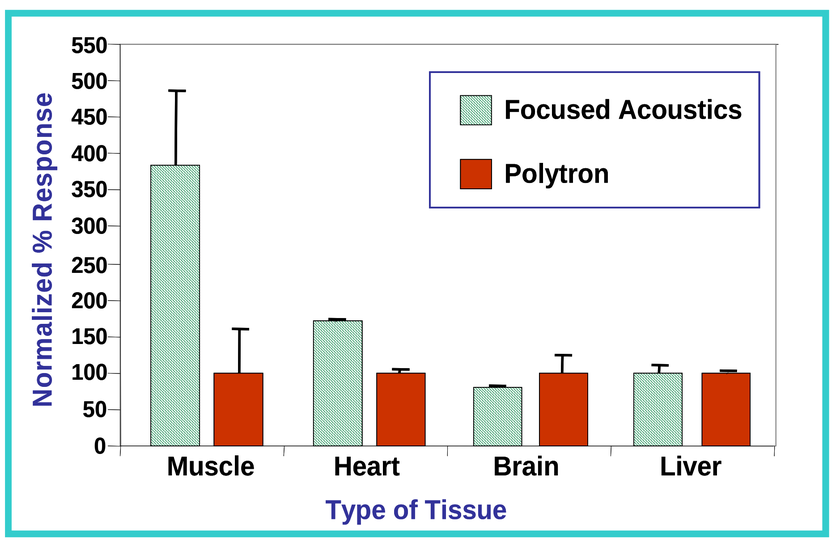

Figure 6. Chart displaying normalized percent response representing recovery of *Drug Compound B* using either Focused Acoustics or Polytron tissue homogenization from different rat tissue types (*n=3* for each); responses represent peak areas measured using LC/MS/MS analysis

Conclusions

Overall, use of automated Focused Acoustics for tissue homogenization resulted in improved extraction efficiency of drug compounds from tissue samples, especially the more difficult to process tissue types, compared with Polytron homogenization, along with a simplified workflow, ease of use, and greater potential for increasing throughput resulting from automated sample processing.

Acknowledgements:

Our group would like to thank James Laugharn at *Covaris, Inc.* for assistance in using and optimizing the *Covaris E200* Focused Acoustics system for our applications.