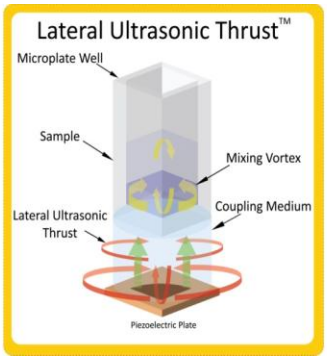
MICROSONIC SYSTEMS™

Improving Triolein Measurement Accuracy in Mass Spectrometry Assays by Using the HENDRIX SM100 Ultrasonic Fluid Processor and RapidFire® Technology

Introduction

Development of therapeutics for obesity is the focus of many pharmaceutical research and development programs, particularly the identification of potential inhibitors of the triacylglycerol (TAG) synthesis pathway. The reaction catalyzed by the enzyme diaceylglycerol aceyl transferase (DGAT) in the TAG pathway produces triolein, and because triolein remains in the lipid bilayer of the cell membrane rather than in the assay buffer making it difficult to accurately measure the compound activity and to reliably detect triolein in the mass spectrometer.

In the protocol described in this note, we used the Microsonic Systems HENDRIX SM100 ultrasonic fluid processor to release triolein from the membrane bilayer. As shown in **Figure 1**, the HENDRIX SM100 uses Lateral Ultrasonic Thrust™ (LUT) technology to create a highly controllable vortex inside an assay well which keeps triolein freely in solution.



◄ **Figure 1**. Microsonic Systems' proprietary Lateral Ultrasonic Thrust™ (LUT) technology creates a controllable vortex inside the sample well and releases triolein from the cell membranes.

The LUT technology utilizes a micro-electro-mechanical systems (MEMS) based transducer and novel Fresnel annular sector actuator array elements to generate bulk acoustic waves which prepare samples and process fluids rapidly and homogenously.

We then used the BIOCIUS RapidFire® Mass Spectrometry (RF-MS) platform to accurately detect and quantify native compounds at the high-throughput speeds required to analyze the large sample numbers in an HTS campaign (**Figure 2**).

We compared the results of various HENDRIX SM100 settings to determine the optimal operating conditions and achieved a 50% improvement in the triolein signal-to-noise ratio in the RF-MS assay.

◄ **Figure 2**. The RapidFire System introduces samples directly to the MS at a rate of approximately six seconds per sample, enabling high-throughput, direct detection of native molecules.

Methods

Triolein in 50 mM Tris buffer with 1% formic acid was added to Nunc 384 polypropylene plates at 50 µL per well. In each plate, enzymes and cell membranes were added to half of the wells ("+enzyme wells"), leaving the other half of the plate as control wells ("-enzyme wells"). One plate was dedicated to each of the HENDRIX SM100 testing conditions (see **Table 1**) and one plate received no treatment at all.

Each plate was then analyzed by RF-MS, and the individual plate signal ratio was calculated as follows: plate signal = (average "+enzyme wells" signals)/ (average "-enzyme wells" signals). The treatment % triolein signal ratio improvement = [(treated plate signal) – (no treatment plate signal)]/(no treatment plate signal) x 100%.

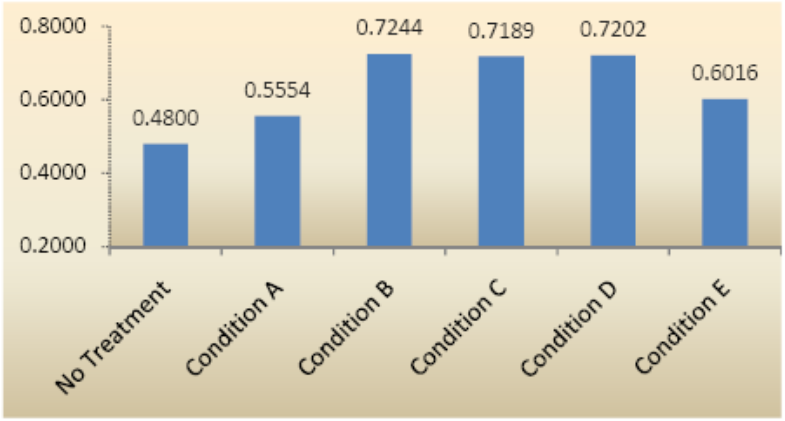
Results

Figure 3 shows the triolein MS signal ratio of control plate and the various experiment plates, and **Figure 4** shows the % triolein signal ratio improvement after ultrasonic fluid processing.

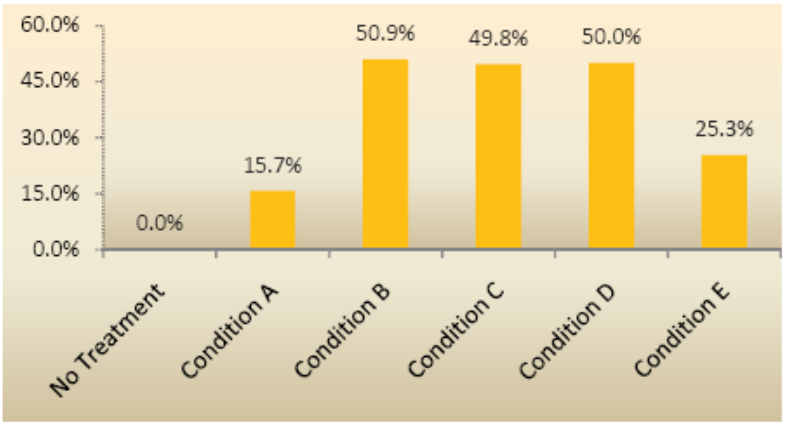
Among the five HENDRIX SM100 settings, conditions B, C and D have the most impact on triolein signal ratios in the MS assays.

Table 1. HENDRIX SM100 settings for Triolein separation tests.

Condition	HENDRIX SM100 Settings
A	2s On/2s Off, 5 cycles, 3000 Hz, 20 V, 20% duty cycle
B	2s On/2s Off, 5 cycles, 3000 Hz, 20 V, 50% duty cycle
C	2s On/2s Off, 5 cycles, 3000 Hz, 25 V, 50% duty cycle
D	2s On/2s Off, 5 cycles, 5000 Hz, 25 V, 50% duty cycle
E	2s On/2s Off, 5 cycles, 3000 Hz, 30V, 50% duty cycle



► **Figure 3. Triolein MS Signal Ratio**. If all of the triolein molecules are floating freely in the assay buffer and not bound to membranes, the signal ratio would be 1. Without treatment, the signal ratio was 0.48; with treatments (conditions A, B, C, D and E), the signal ratio increased substantially. The highest signal ratios, at approximately 0.72, were measured in conditions B, C and D.



► **Figure 4. % Triolein Signal Ratio Improvements**. Compared to the control plate (no treatment), plates processed with the HENDRIX SM100 showed improvements in the triolein signal ratio. In conditions B, C and D, the %signal ratio improvements are 50.9%, 49.9% and 50.0%, respectively.

Conclusion

The gentle vortex generated by the HENDRIX SM100 significantly improves triolein measurement accuracy in MS assays. By using the HENDRIX SM100 for ultrasonic fluid processing, triolein molecules are released from the membranes in the assay resulting more accurate assay results. Analysis by RF-MS enables accurate detection of native molecules at unprecedented speeds. The combination is specifically effective for lipidic molecules and other compounds that are difficult to analyze with conventional methods.

Ordering Information

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