Application Note

Analysis of lipids and free fatty acids

Introduction

Lipids play multiple key roles in biological systems, including acting as secondary energy reserves, as components of membranes and participating in cell-signalling pathways. Aberration in levels of lipid and/or free fatty acids have been linked to several diseases. Consequently, the detailed analysis of lipids found in biological samples is crucial for our understanding of disease mechanisms, progression, and development of treatment strategies. Heat stabilization using the Stabilizor™ system is an effective way of preventing changes in levels of lipids and free fatty acids which have been shown rapidly post-sampling.

Technological benefits

- Preserves the *in vivo* levels of lipids and other unstable biomarkers, increasing the accuracy of quantification analysis
- Retains the *in vivo* levels of proteins, peptides and modifications from the moment of sampling throughout the analysis workflow, increasing the precision of analysis
- Additive-free method, for biofluids and all tissue types, both fresh or frozen
- Ensures quality and standardization of sample collection

The effect of heat stabilization on Sphingolipid regulation analysis, heat stabilized vs. snap frozen samples

Sphingolipds and their phospho-forms are key regulators of cell death and survival. They play an important role as part of the cell membrane and as signaling molecules orchestrating cell growth, differentiation and apoptosis.

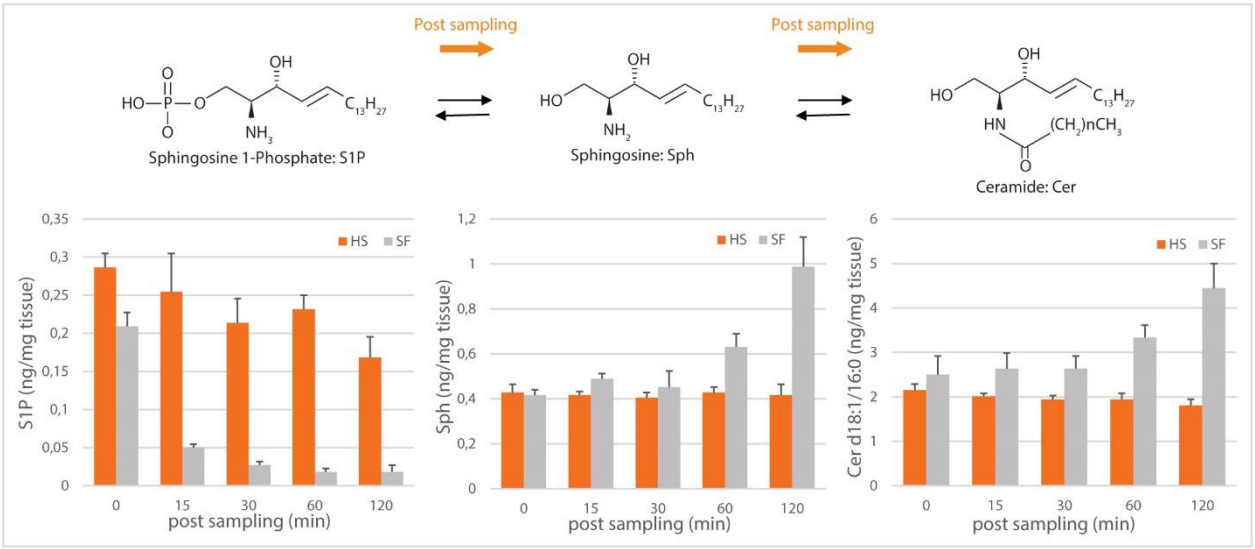

Figure 1. LC-MS determination of sphingolipids and their levels in liver in room temperature , Heat stabilized (HS, orange) samples are compared to snap frozen (SF, gray) up to 120 minutes.

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The results indicate a time dependent shift in the snap frozen samples from Phosphorylated Sphingosine (S1P) into the non-phosphorylated and apoptosis associated forms Sphingosine (Sph) and Ceramide (Cer). The heat stabilized samples remain in most forms stable over the time course.

Levels of free fatty acids in tissue homogenates, heat stabilized vs. snap frozen samples

Post-sampling effects due to tissue thawing and sample preparation induce massive release of free fatty acids due to the continued activity of enzymatic actions in snap frozen tissue.

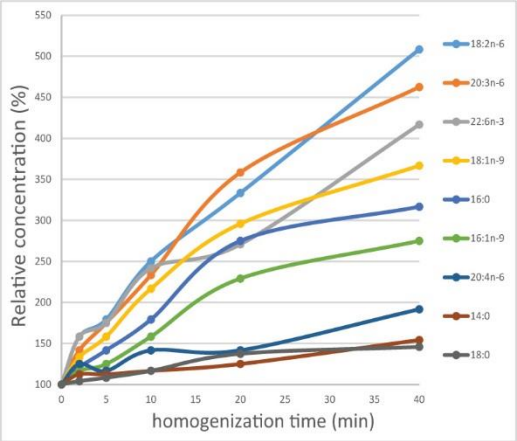

Figure 2. The effect of homogenization time at room temperature on FFA levels in a snap frozen brain. To confirm the effects of ongoing phospholipase activity a homogenate from frozen brain tissue was left at room temperature for 0–40 min before protein precipitation and analysis. All analyzed FFAs increased in concentration over time. Statistical analysis by treating all FFAs as dependent variables revealed a significant increase already at 2 min, which continued over time.

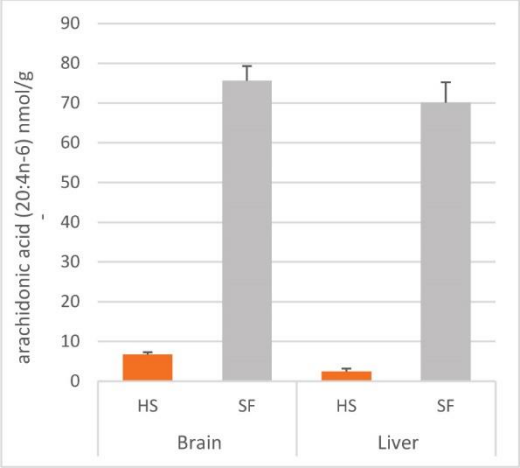

Figure 3. Effects of heat stabilization of brain and liver tissue on detected FFA concentrations. Post-sampling effects due to tissue thawing and sample preparation induced a massive release of FFAs from non-stabilized liver and brain tissues compared to heat stabilized tissue due to continued lipase activity.

References

- Jernerén, F., et al., Post-sampling release of free fatty acids—effects of heat stabilization and methods of euthanasia, Journal of Pharmacological and Toxicological Methods (2015), 71:13-20
- Saigusa, D., et al., Simultaneous Quantication of Sphingolipids in Small Quantities of Liver by LC-MS/MS, Mass Spectrom (Tokyo) 2014; 3(4): S0046