OTHER

THE PRECIPITATION OF PROTEINS AND LIPID EXTRACTION FROM COMPLETELY HOMOGENIZED RAT SKIN TISSUES

/ CONTEXT

In this study, protein precipitation and bioactive lipid extraction from rat skin tissues is reported. The combination Solid Phase Extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS) approach was used to measure concentrations of lipid mediators in these tissues. Rat skin tissues were homogenized using CKMix50_7mL Precellys® Lysing Kit and Precellys® Evolution tissue homogenizer combined with Cryolys® cooling unit. In addition, temperatures of homogenates were measured to investigate if temperature of complete homogenate of rat skin tissues remained below 40°C.

/ MATERIALS

- **Automated Homogenizer**: Precellys® Evolution and Cryolys® cooling unit
- **Lysing Kits**: Tissue grinding CKMix50_7mL (Cat #: KT03961-1-306.7)
- **Tissue Samples**: Rat skin tissues (dorsal and ventral hindpaw)
- **Homogenization Buffer**: Dry ice-cold methanol

/ PROTOCOL

Samples: A total of 20-90 mg of adult rat skin tissues (dorsal and ventral) were obtained following standard practice and stored at -80°C until use.

Homogenization: Tissues were homogenized in 500 µL methanol in 7 mL Precellys® tubes containing a mix of 2.8 mm and 5.0 mm ceramic beads. Tissues were homogenized by running 5 cycles of 10 sec at 8,000 rpm, with a 120 sec break between cycles.

Cryolys® cooling unit: Samples were homogenized once the temperature of cooling unit homogenization chamber reached 5°C.

Temperature measurements: Immediately after sample homogenization, sample temperatures were measured using temperature probe.

/ RESULTS

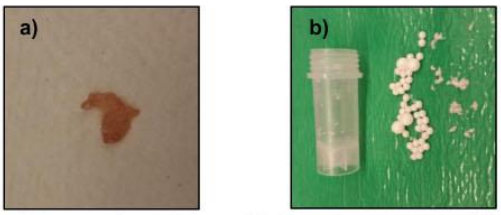

Fig. 1 Images of a whole rat hindpaw tissue sample (a), and homogenized tissue (b) using Precellys® Evolution.

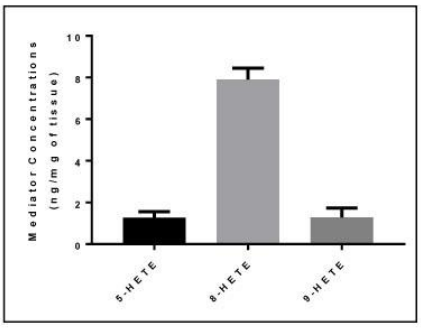

Fig. 2 Bioactive lipid mediators of the Arachidonic acid cascade were successfully measured, by LC-MS/MS, after lipids were extracted from the homogenate by solid phase extraction. Concentrations of 5-HETE, 8-HETE and 9-HETE were measured to be 1.3 ±0.3, 7.9±0.6 and 1.3 ± 0.4 ng/mg of tissue, respectively.

/ CUSTOMER

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/ CONCLUSION

The rat hindpaw skin is considered to have thicker epidermis than the back skin, thus it is more challenging to obtain complete homogenate when working with these tissues. The combination of Precellys® lysing kit matrix and homogenization settings using Precellys® Evolution tissue homogenizer, allowed for the successful generation of a rat hindpaw whole tissue sample homogenate. The study also showed that Cryolys® cooling unit was able to maintain sample temperatures below 40°C.

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