**Oxylipins/Endocannabinoids analysis protocol**

*UPLC-MS/MS analysis* – Analytical targets were quantified using internal standard methodology against authentic calibration standards detected by electrospray ionization with positive/negative switching and multiple reaction monitoring on a API 6500 QTrap (AB Sciex, Framingham, MA, USA). Briefly, samples were re-randomized for acquisition (See Table 2, "Run order"), with Blanks, UTAKs and calibration sets scattered regularly throughout the set. For analysis 5 µL of the extract were injected and separated using a Shimadzu Nexera X2 UPLC (Shimadzu, Kyoto, Japan) with an Acquity UPLC BEH C18 1.7µm 2.1 × 100 mm column (Waters, Milford, MA, USA) and a solvent gradient using modifications of a previously published protocol for oxylipins and endocannabinoids *[1]* detailed in table 1. Samples were held at 10 °C. Separated residues were detected by positive/negative mode switching, with negative mode electrospray ionization for oxylipins and nitro lipids and positive mode electrospray ionization for endocannabinoids and fatty acids using scheduled multiple reaction monitoring on an API 6500 QTrap (AB Sciex, Framingham, MA, USA). Analytes were quantified using internal standard methods and 6 to 10 point calibration curves (r2 ≥ 0.997) with the internal standard used to quantify the extraction surrogate recovery and to establish relative retention times. Calibrants and internal standards were either synthesized [10,11-DHHep, and PHAU] or purchased from Cayman Chemical (Ann Arbor, MI), Medical Isotopes (Pelham, NH) or Avanti Polar Lipids Inc. (Alabaster, AL), unless otherwise indicated. Larodan Fine Lipids (Malmö, Sweden) provided the linoleate derived triols 9,12,13-TriHOME, 9,10,13-TriHOME, 9_10-EpO and 9,10-DiHHex. Data was processed utilizing AB Sciex MultiQuant v 3.0.1. Auto-integrations were manually inspected and corrected if necessary. Peaks areas were quantified using response ratios incorporating surrogate peak responses. The obtained peak areas of targets were corrected by appropriate internal standards (ISTD) and concentrations (in nM) were calculated by referring to calibration curves.

**Table 1: solvent gradient for oxylipins and endocannabinoids separation**

| Time after injection (min) | 0.1% acetic acid | 90:10 acetonitrile:isopropanol v/v |
|---|---|---|
| 0.00 | 75% | 25% |
| 1.00 | 60% | 40% |
| 2.50 | 58% | 42% |
| 4.50 | 50% | 50% |
| 10.50 | 35% | 65% |
| 12.50 | 25% | 75% |
| 13.25 | 20% | 80% |
| 17.25 | 15% | 85% |
| 18.25 | 5% | 95% |
| 18.75 | 0% | 100% |
| 19.00 | 0% | 100% |
| 19.10 | 75% | 25% |
| 20.00 | stop | |

**References:**


*1.* Agrawal, K., L.A. Hassoun, N. Foolad, *T.L. Pedersen*, R.K. Sivamani, J.W. Newman. 2017. Sweat lipid mediator profiling: a non-invasive approach for cutaneous research. *J. Lipid Res.* 58:188–195 [EPub: Nov 7, 2016]. doi: 10.1194/jlr.M071738