Plasma free Oxylipins/Endocannabinoids analysis protocol

Oxylipins and endocannabinoids extraction – After sample randomization, 50 µl plasma aliquots were enriched with deuterated surrogates in 10 µL methanol (Table S1 from *[1]*) and 5 µl of BHT/EDTA in 1:1 methanol/water (v/v). A total of 200 µl 1-cyclohexyl uredio, 3-dodecanoic acid / 1-phenyl ureido, 3-hexanoic acid (CUDA / PUHA) in 1:1 methanol:acetonitrile was added. Protein precipitate and debris were removed by centrifugation for 10 min at 4,500 RCF. The supernatant was filtered by centrifugation through 0.2 µm PVDF membranes (Agilent Technologies, Santa Clara, CA, USA) at 500 RCF for 5 min.

UPLC-MS/MS analysis for oxylipins and endocannabinoids– 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, 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 ($r2 \geq 0.997$) 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 PUHA] or purchased from Cayman Chemical (Ann Arbor, MI), Medical Isotopes (Pelham, NH), Avanti Polar Lipids Inc. (Alabaster, AL), or Larodan Fine Lipids (Malmö, Sweden). Data was processed with AB Sciex MultiQuant v 3.0.1. Auto-integrations were manually inspected and corrected as 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 nM concentrations 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. K. Agrawal, 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