Lipid analysis in salmon muscle and liver tissue

**LC CONDITIONS**

**LC system:** Acquity Waters UPLC system consisted of Sample manager, Column manager and Binary Solvent manager.

**Lock mass:** Leucine-Enkephaline; Lock Mass correction applied simultaneously with data acquisition. Data were processed with Progenesis.

**UPLC column:** Acquity UPLC CSH C18 2.1x100 mm, 1.7um (+ guard column)

**Column temp.:** 55°C

**Flow rate:** 0.35 ml/min

**Mobile phase A:** Acetonitrile/water (60:40, v/v) with 10mM ammonium formate and 0.1 % formic acid

**Mobile phase B:** Isopropanol/acetonitrile (90:10, v/v) with 10mM ammonium formate and 0.1 % formic acid

**Weak wash:** Isopropanol/acetonitrile (90:10, v/v)

**Injection:** Lipid extracts were diluted with a mixture of isopropanol/acetonitrile/water (2:1:1, v/v/v) prior injection.

Muscle extracts – diluted 20x, injection volume 4uL

Liver extracts – diluted 5x, injection volume 5 uL

*Table 1 - Gradient*

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>%A</th>
<th>%B</th>
<th>Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>60</td>
<td>40</td>
<td>Initial</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>43</td>
<td>6</td>
</tr>
<tr>
<td>2,1</td>
<td>50</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>46</td>
<td>54</td>
<td>6</td>
</tr>
<tr>
<td>12,1</td>
<td>30</td>
<td>70</td>
<td>1</td>
</tr>
<tr>
<td>18</td>
<td>1</td>
<td>99</td>
<td>6</td>
</tr>
<tr>
<td>22,5</td>
<td>1</td>
<td>99</td>
<td>6</td>
</tr>
<tr>
<td>22,6</td>
<td>60</td>
<td>40</td>
<td>6</td>
</tr>
<tr>
<td>25</td>
<td>60</td>
<td>40</td>
<td>6</td>
</tr>
</tbody>
</table>

**MS conditions**

**Mass spectrometer:** Synapt G2 HDMS

**Acquisition mode:** MSe (Transfer MS collision energy ramped from 15 to 35 eV in POS mode and from 15 to 40 eV in NEG mode)

**Ionization mode:** ESI positive/negative

**Capillary voltage:** 2.8 kV/-1.9kV

**Cone voltage:** 30V

**Desolation temp.:** 550°C

**Desolation gas:** 900L/Hr

**Source temp.:** 120°C

**Acquisition range:** 50 to 1999 m/z

**Software**

MassLynx V4.1 – instrument control, data acquisition and processing (Lock mass correction).

Progenesis QI – MS data processing
SAMPLE TREATMENT

Sample: Salmon muscle or liver tissue (50 mg), stored at -80°C.
Homogenizer: Precellys®24 equipped with temperature controller Cryolys; Precellys brand tubes (2 mL), Zirconium oxide beads with diameter 1.4 mm (all Bertin Technologies).
Lab equipment: Centrifuge (MiniSpin, Eppendorf), Shaker (Thermal shake lite, VWR), Syringe filters with GHP membrane, 0.2 um, Ø 13 mm (Acrodisc®, Pall Laboratory).
Chemicals: Chloroform, methanol, acetic acid (20mmol/L) - all used solvents were kept in ice.

Extraction procedure

- Weight accurately 50 mg of tissue into a 2 mL tube (Precellys brand) pre-filled with 0.5±0.01g zirconium oxide beads; the tissue was kept frozen during cutting and weighing.
- Add 500 ul of a cold mixture of chloroform/methanol (2:1, v/v) and homogenize the sample using Precellys 24 (for detailed settings see Table 2).
- Add 500 ul of a cold mixture of chloroform/methanol (2:1, v/v) to the tube and shake for 10 min (1500 rpm, 16°C).
- Phase separation is induced by adding 500 ul of 20mM Acetic acid.
- After 10 minutes of vortexing (1500 rpm, 16°C) the tube was centrifuged at max. speed (6 min, 13 400 rpm).
- Collect 400 uL of lower (organic) layer.
- Filtrate the resulting extracts through a 0,2um filter and keep them in dark glass vials (use lids lined with PTFE).
- Store lipid extracts at -20°C until further analysis.
- Samples were diluted with a mixture of isopropanol/acetonitrile/water (2:1:1, v/v/v) prior injection.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Power (rpm)</th>
<th>Time</th>
<th># of cycles</th>
<th>Pause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>6500</td>
<td>20 s</td>
<td>2</td>
<td>15 s</td>
</tr>
<tr>
<td>Muscle</td>
<td>6500</td>
<td>30 s</td>
<td>3</td>
<td>15 s</td>
</tr>
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