UHPLC-IMS-Q-ToF-MS analysis of Maradolipids, found exclusively in *Caenorhabditis elegans* dauer larvae

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• *Caenorhabditis elegans* research
  • Analytical Method development
    – Lipid analysis methods
    – Sample preparation
    – “Single Worm” methods

• “Decoding” of the *C. elegans* metabolome & lipidome
• Regulation of lipid metabolism
• Metabolic reconstructions, flux balance analysis and novel metabolic pathways
• *Caenorhabditis elegans* research
  • ~ 1 mm x 65 µm size
  • 959 (hermaphrodite)/1031 (male) cells
  • 2-3 weeks lifespan
  • Majority of human diseases genes and pathways are present in *C. elegans*
  • Model system for …
    – development
    – neurobiology
    – host-pathogen/microbe interactions
    – ageing
    – alzheimer
    – nutrition & diabetes
• *Caenorhabditis elegans* research

| Organism                          | Advantages / Disadvantages                                                                 |
|----------------------------------|-------------------------------------------------------------------------------------------|
| *Mus musculus* mouse             | (+) Genome is available
|                                  | (+) Strong genetic, physiological overlap with humans
|                                  | (-) Ethical concerns
|                                  | (-) Expensive
|                                  | (-) Long generation time (2-3 months)
|                                  | (-) Long Lifespan
|                                  | (-) Not amenable to high-throughput screens                                                |
| *Drosophila melanogaster* fruit fly | (+) Inexpensive/easy to grow
|                                  | (+) Genome is available
|                                  | (+) Straightforward genetic tools exist
|                                  | (+) Short generation time (~ 10 days)
|                                  | (+)/(−) 50-80% of fly genes homologous to human genes
|                                  | (-) Mutants cannot be frozen                                                               |
| *Caenorhabditis elegans* roundworm | (+) Inexpensive/easy to grow
|                                  | (+) Genome is available
|                                  | (+) Straightforward genetic tools exist
|                                  | (+) **Short generation time (2-3 days)**
|                                  | (+) Short lifespan (2-3 weeks)
|                                  | (+) Small, exactly 959 somatic cells
|                                  | (+) Invariant development
|                                  | (+) Transparent
|                                  | (+) Has organs/differentiated tissues
|                                  | (+) Mutants can be frozen
|                                  | (+)/(−) 50-80% of worm genes homologous to human genes                                    |
Primer on lipid(ome) analysis

*LC-IMS-MS based lipid analysis*

- Agilent 6560 IMS-Q-ToF-MS
Primer on lipid(ome) analysis

**LC-IMS-MS based lipid analysis**

- **UHPLC-IMS-ToF-MS**
- **RP-UPLC-UHR-ToF-MS** (Witting et al., 2014)
  - Agilent 1290 UHPLC & 6560 IMS-Q-ToF-MS
  - Waters Cortecs C18, 150 mm x 2.1 mm ID
  - A: 60% ACN / 40% H₂O + 10 mM NH₄OOCH + 0.1% HOOCH
  - B: 90% iPrOH / 10% ACN + 10 mM NH₄OOCH + 0.1% HOOCH

- Data Dependent Acquisition (QToF only)
- Data Independent Acquisition (AllIons)

- (+) / (-) ionization mode

- Extraction using Folch,MTBE, Bligh & Dyer or BUME method
- Fractionation of lipids (Bodennec et al, 2000)

Witting et al., 2014, Witting & Schmitt-Kopplin, 2013
Primer on lipid(ome) analysis

LC-IMS-MS based lipid analysis
Characterization of maradolipids in *C. elegans*

**Allions fragmentation combined with ion mobility**

**LC-IMS-MS based characterization of maradolipids in *C. elegans***

- *Caenorhabditis elegans* is a major model organism in biomedical research
- It develops through different larval stages to reproductive adults
- Upon food scarcity L1 larvae can enter an alternative state, called „dauer“
- Dauer larvae are resistant to harsh environmental conditions and are non-feeding
- Dauer larvae produce glycolipids found only in this development stage called maradolipids
- Maradolipids have been so far only analyzed by shotgun lipidomics

![Diagram showing the development stages of *C. elegans* and the role of Dauer larvae](image)
Characterization of maradolipids in *C. elegans*

*Alllons fragmentation combined with ion mobility*

- Characterization of maradolipid standards

Witting et al., 2020, under review
Characterization of maradolipids in *C. elegans*

*Allions* fragmentation combined with ion mobility

### Characterization of maradolipid standards

| Name           | adduct           | m/z    | CCS +/- SD (multi field) | CCS +/- SD (single field) | RT +/- SD |
|----------------|------------------|--------|--------------------------|---------------------------|-----------|
| Mar(14:0/14:0) | [M+NH4]^+        | 780.5467 | 282.87 +/- 0.25          | 284.00 +/- 0.26 (-0.40 %) | 12.98 +/- 0.03 |
| Mar(15:0/15:0) |                  | 808.578  | 289.13 +/- 0.21          | 290.60 +/- 0.20 (-0.51 %) | 13.90 +/- 0.02 |
| Mar(14:0/18:1) |                  | 834.5937 | 293.00 +/- 0.20          | 294.20 +/- 0.26 (-0.41 %) | 14.38 +/- 0.02 |
| Mar(16:0/16:0) |                  | 836.6093 | 294.93 +/- 0.25          | 296.57 +/- 0.38 (-0.55 %) | 15.10 +/- 0.02 |
| Mar(15:0/17:0) |                  | 836.6093 | 294.93 +/- 0.25          | 296.63 +/- 0.15 (-0.58 %) | 15.47 +/- 0.02 |
| Mar(15:0/18:1) |                  | 848.6093 | 296.03 +/- 0.23          | 297.37 +/- 0.35 (-0.45 %) | 14.78 +/- 0.02 |
| Mar(16:0/18:1) |                  | 862.625  | 298.77 +/- 0.25          | 300.70 +/- 0.26 (-0.65 %) | 15.52 +/- 0.02 |
| Mar(17:0/18:1) |                  | 876.6406 | 301.67 +/- 0.15          | 303.17 +/- 0.23 (-0.50 %) | 15.85 +/- 0.02 |
| Mar(18:1/18:1) |                  | 888.6406 | 302.87 +/- 0.32          | 304.20 +/- 0.00 (-0.44 %) | 15.56 +/- 0.02 |
| Mar(18:1/19:1) |                  | 902.6563 | 306.80 +/- 0.17          | 307.67 +/- 0.21 (-0.28 %) | 16.23 +/- 0.02 |

Witting et al., 2020, under review
Characterization of maradolipids in *C. elegans*

*Allons fragmentation combined with ion mobility*

- Characterization of maradolipid standards
- m/z, kendrick mass defect, retention time and CCS values can be used to filter potential candidates

Witting et al., 2020, under review
Characterization of maradolipids in *C. elegans*

**AllIons fragmentation combined with ion mobility**

- **Characterization of maradolipid standards**
- Fragmentation of maradolipid standards was studied using AllIons fragmentation
  - 10, 20 and 40 eV were used
  - Fragments include...
    - ... fatty acyls
    - ... trehalose fragments
    - ... loss of fatty acyl
  - [Trehalose-H$_2$O-H]$^-$ and [Trehalose-2H$_2$O-H]$^-$ are fragments that can be used to screen for maradolipids

Witting et al., 2020, under review
Characterization of maradolipids in *C. elegans*

**Allions fragmentation combined with ion mobility**

- Screening for maradolipid
- Use of all Allions and m/z 305 and 323 can be used to search for new maradolipids

Witting et al., 2020, under review
Characterization of maradolipids in *C. elegans*

**AllIons fragmentation combined with ion mobility**

Analysis of *C. elegans* dauer larvae

- Typical fragments from maradolipids are m/z 323 and 305 derived from trehalose

- The high collision energy channel was searched for co-elution of these fragments

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Characterization of maradolipids in *C. elegans*

*Allions* fragmentation combined with ion mobility

- Analysis of *C. elegans* dauer larvae

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