# SUPPORTING INFORMATION

Kwang-Youl Kim[1], Hyoe-Jin Joo[1], Hye-Won Kwon[2], Heekyeong Kim[1], William S. Hancock[1, 3], and Young-Ki Paik[1, 2]

[1]Department of Biochemistry, College of Life Sciences and Biotechnology, Yonsei Proteome Research Center, Department of Biomedical Science, and [2]Department of Integrated Omics for Biomedical Research, World Class University Program, Yonsei University, Seoul, Korea; [3]Barnett Institute, Department of Chemistry, Northeastern University, Boston, MA, USA

Development of a Method to Quantitate Nematode Pheromone for Study of Small-Molecule Metabolism in *Caenorhabditis elegans*

ABSTRACT

Pheromones produced by *Caenorhabditis elegans* are considered as key regulators of development, mating, and social behaviors in this organism. Here, we present a rapid

mass spectrometry-based method (PheroQu) for absolute quantitation of nematode pheromones (e.g., daumone 1, 2, and 3) both in *C. elegans* worm bodies (as few as 20 worms) and in liquid culture medium. Pheromones were separated by ultra performance liquid chromatography and monitored by a positive electrospray ionization detector in the multiple-reaction monitoring mode. The *daf-22* mutant worms were used as surrogate matrix for calibration, and stable deuterated isotope-containing pheromone was used as internal standard for measuring changes in pheromones in N2 wild type and other strains under different growth conditions. The worm-body pheromones were extracted by acidified acetonitrile solvent, and the secreted pheromones were extracted from culture medium with solid-phase extraction cartridges. The run time was achieved in less than 2 min. The method was validated for specificity, linearity, accuracy, precision, recovery, and stability. The assay was linear over an amount range of 2–250 fmol, and the limit of quantitation was 2 fmol for daumone 1, 2, and 3 in both worm bodies and culture medium. With the PheroQu method, we were able to identify the location of pheromone biosynthesis and determine the changes in different pheromone types synthesized, according to developmental stages and aging process. This method, which is simple, rapid, sensitive, and specific, will be useful for the study of small-molecule metabolism during developmental stages of *C. elegans*.

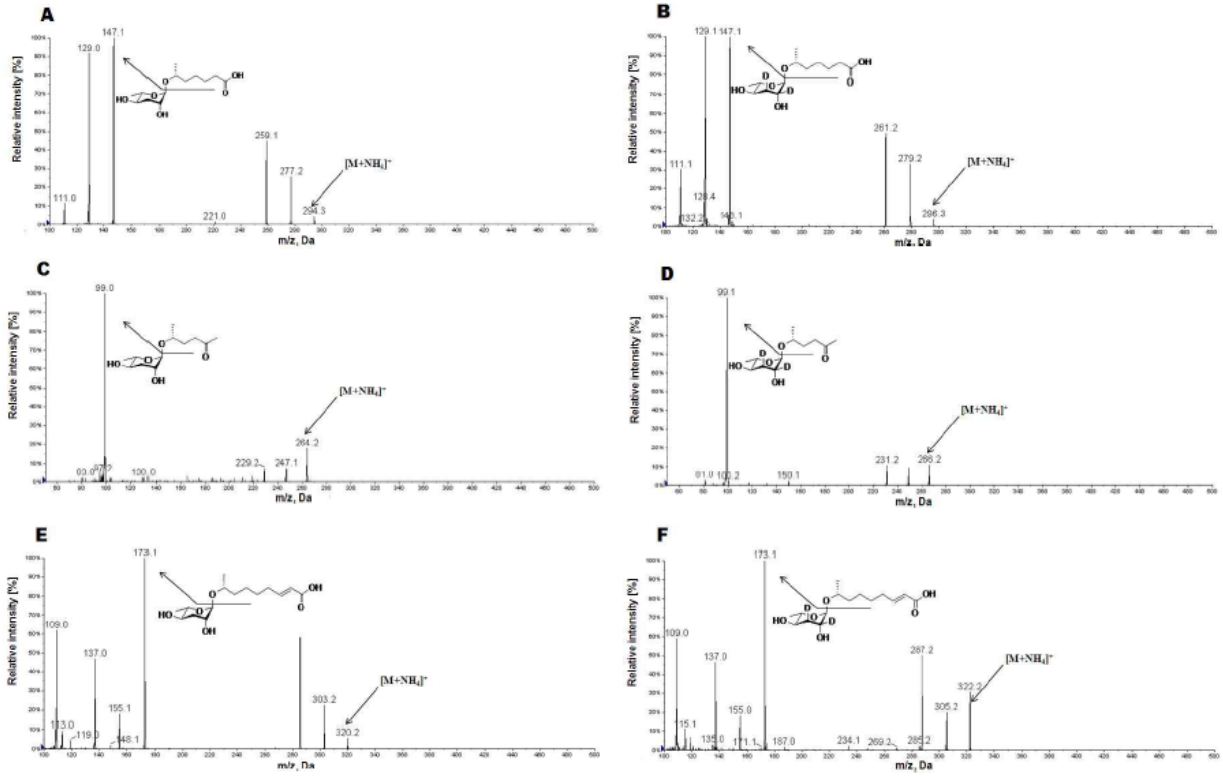


**Figure S-1.** Collision-induced dissociation mass spectrum of ammonium-adduct $[M+NH_4]^+$ ions of daumones and internal standards in positive-ion electrospray ionization (ESI). Product-ion spectrum from **a)** daumone 1, **b)** deuterated (D2)-daumone 1, **c)** daumone 2, **d)** D2-daumone 2, **e)** daumone 3, and **f)** D2-daumone 3, obtained by fragmentation of the $(M+NH_4)^+$ ion at each *m*/*z*, using a collision potential of 12 eV. For ESI experiments, all samples (1 pmol/µl) were diluted in solvent (2 mM $NH_4Ac$ in 50% v/v acetonitrile containing 0.1% v/v formic acid) and infused into the mass spectrometer at a flow rate of 10 µl/min. The multiple reaction monitoring transitions of daumones and D2 internal standards that produced the highest product-ion signals (arrows) were used for daumone quantitation.

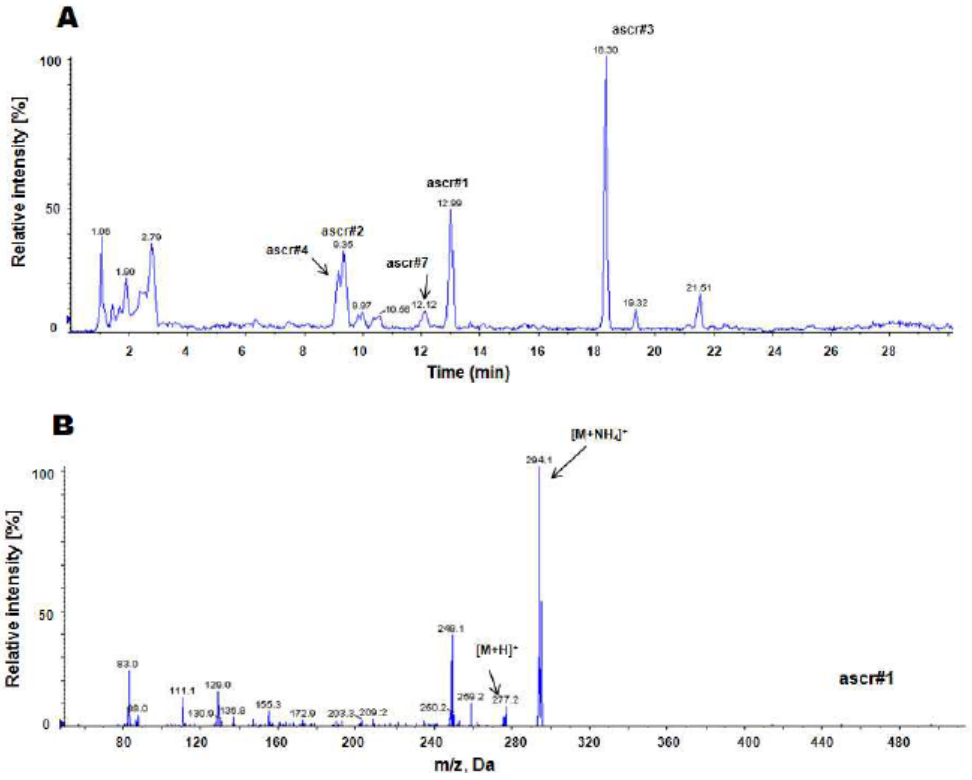


**Figure S-2.** LC-MS total ion current chromatogram from the neutral loss scan experiment. **A)** N2 worm pellets were analyzed by positive ion LC-MS/MS in neutral loss scan, and ascarosides species were detected as $[M+NH4]^+$ ions by neutral loss scanning of 147.2 Da (ascarylose moiety). **B)** MS/MS spectra of ascr#1 gave fragment ions containing the $[M+H]^+$ at 277.1, corresponding to ascr#1 $[M+NH4]^+$. Ascr#1, daumone 1; ascr#2, daumone 2; ascr#3, daumone 3, ascr#4, daumone 4; ascr#7, daumone 7.

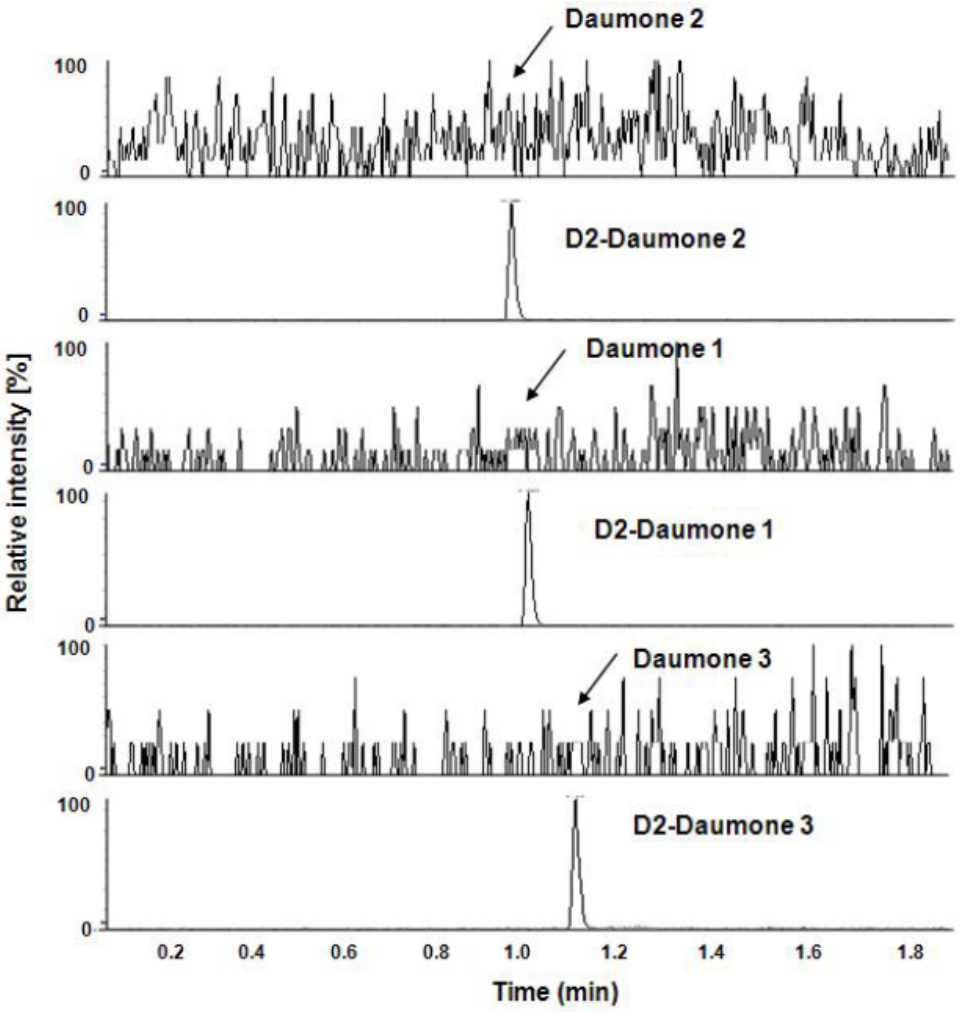


**Figure S-3.** Representative chromatograms of daumones and deuterated (D2) internal standards in blank sample (*daf-22* mutant worm-spiked internal standard). Black arrows indicate retention time (min) of each pheromone detected.

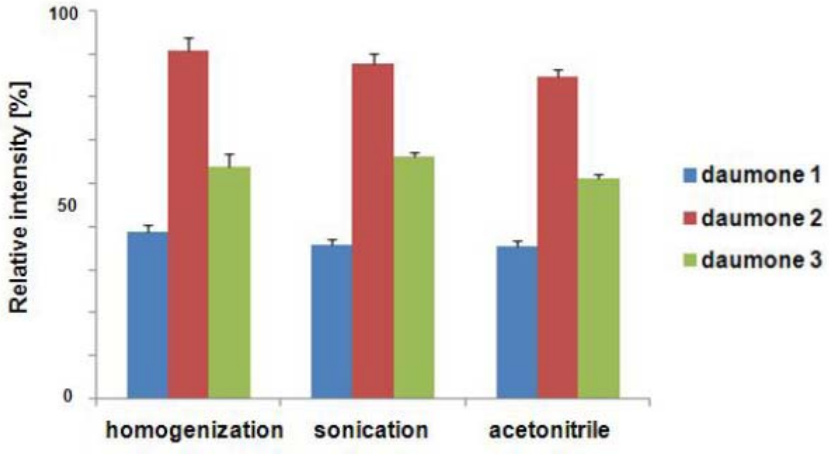


**Figure S-4.** Comparison of different extraction methods for daumones from worm bodies. Homogenization, sonication, and extraction with organic solvent were investigated. Worm pellets (20 µl containing approximately 1,000 worm bodies) were subjected in parallel to the indicated methods in three separate trials. Bars represent mean values for each trial (N=3) plus 1 standard error.

**Table S-1.** Stability of daumones under various laboratory conditions.

| Stability Experiments | Storage condition | Nominal amt.(fmol) | Calculated amt.(fmol) | CV (%) | Change (%) |
|---|---|---|---|---|---|
| **1) Daumone 1** | | | | | |
| Bench-top stability | 4°C, 2 weeks | 3 | 2.64 | 1.10 | −1.26 |
| in stock solution | | 80 | 82.40 | 3.55 | 13.37 |
| Post-preparative stability | Autosampler | 3 | 2.98 | 8.38 | 5.29 |
| | (4°C, 24 h) | 80 | 83.10 | 2.20 | 9.39 |
| Short-term stability | RT, 24 h | 3 | 2.95 | 13.65 | 4 |
| | | 80 | 83.47 | 3.68 | 9.87 |
| Long-term stability | 3 months | 3 | 2.83 | 5.85 | −2.8 |
| | at −70°C | 80 | 75.97 | 2.48 | −8.21 |
| **2) Daumone 2** | | | | | |
| Bench-top stability | 4°C, 2 weeks | 3 | 3.04 | 4.64 | −0.55 |
| in stock solution | | 80 | 80.29 | 9.11 | −2.87 |
| Post-preparative stability | Autosampler | 3 | 2.83 | 9.14 | −5.35 |
| | (4°C, 24 h) | 80 | 85.63 | 7.82 | −10.04 |
| Short-term stability | RT, 24 h | 3 | 2.78 | 8.10 | 4 |
| | | 80 | 76.50 | 4.78 | 9.87 |
| Long-term stability | 3 months | 3 | 2.90 | 8.90 | 0.15 |
| | at −70°C | 80 | 76.87 | 2.07 | 2.56 |
| **3) Daumone 3** | | | | | |
| Bench-top stability | 4°C, 2 weeks | 3 | 2.89 | 9.50 | −10.04 |
| in stock solution | | 80 | 79.02 | 5.45 | −2.92 |
| Post-preparative stability | Autosampler | 3 | 2.86 | 7.53 | −1.15 |

| | | | | | |
|---|---|---|---|---|---|
| | (4°C, 24 h) | 80 | 83.37 | 2.58 | 8.46 |
| Short-term stability | RT, 24 h | 3 | 2.90 | 8.61 | 4 |
| | | 80 | 85.93 | 8.73 | 9.87 |
| Long-term stability | 3 months | 3 | 2.90 | 8.90 | −13.57 |
| | at −70°C | 80 | 76.87 | 2.07 | −7.65 |