**Overview**

**Purpose:** To investigate metabolic changes in hermaphroditic C. elegans via isotopic ratio analysis using high-resolution accurate mass mass spectrometry (HRAM) for mass analysis.

**Methods:** IROA (Isotopic Ratio Analysis) combined with HRAM MS/MS was used to investigate changes in metabolic pathways due to sexual maturation in C. elegans. The analysis involved reverse phase (RPLC) chromatography and hydrophilic interaction liquid chromatography (HILIC) methods with high-resolution accurate mass measurements (HRAM). Sample analyses were conducted in both positive and negative ESI modes. HRAM MS/MS data were collected at a mass resolution of 50,900 FWHM.

**Results:** HRAM-MS data of samples were used to identify metabolites in C. elegans. The methods allowed for the identification of both isotopic and non-isotopic species. The HRAM data analysis showed that the mass accuracy of these metabolites was within 1 ppm. Examples of these species included carnitine, ascorbic acid, and other amino acids.

**Conclusions:** The use of IROA-MS/MS with HRAM allowed for the identification of novel metabolites in C. elegans. This method provided a powerful tool for metabolite identification and quantification, enabling researchers to gain deeper insights into the metabolic changes occurring during sexual maturation in this model organism.