SUPPORTING INFORMATION

Figure S1

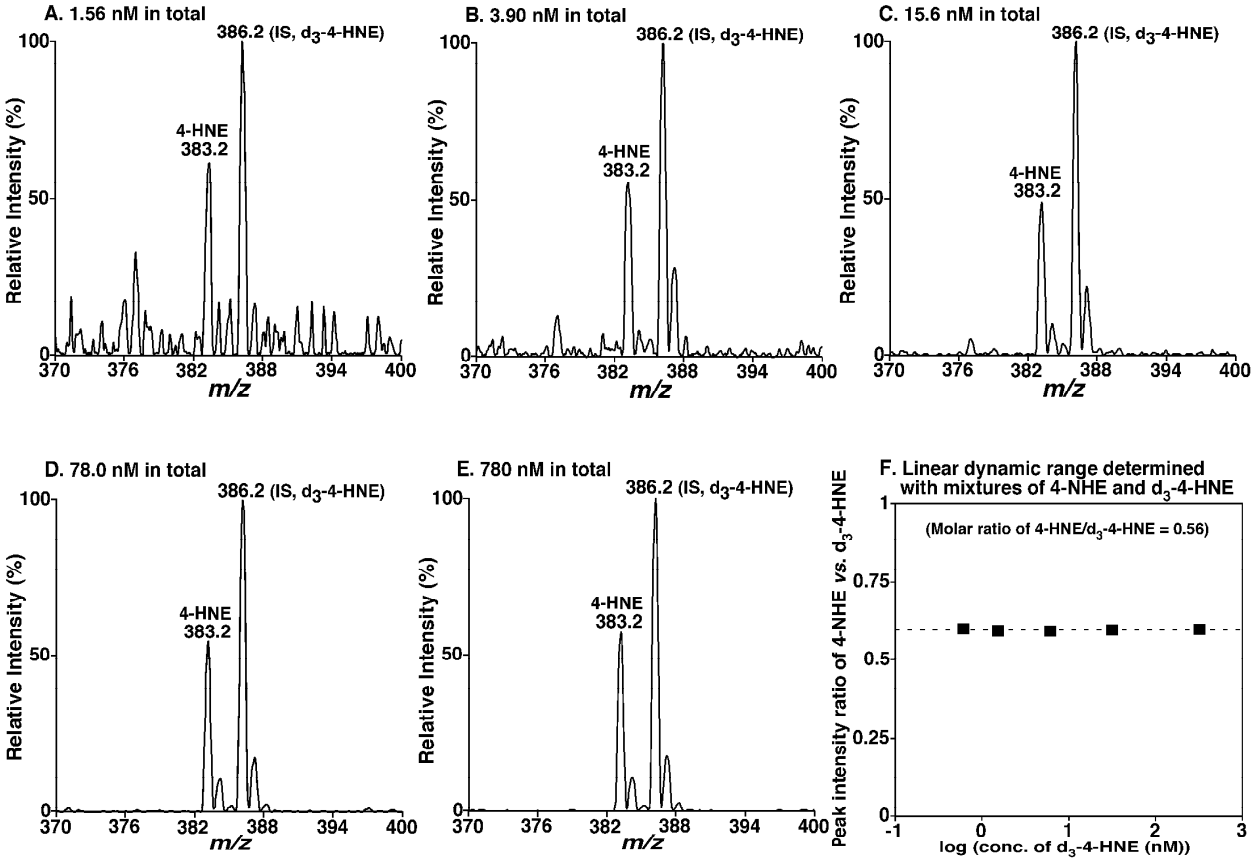

Figure S1. Examination of dynamic range and limit of quantification of the method with mixtures of 4-HNE and d_3-4-HNE at a constant molar ratio, but different concentrations after carnosine derivatization. Carnosine adducts of a mixture of 4-HNE and d_3-4-HNE (a molar ratio of 0.56:1) were prepared by incubating the mixture with carnosine at 37^{o}C for 24 hrs as described under "Materials and Methods". Neutral loss scans of 71.0 Da at different concentrations of 4-HNE and d_3-4-HNE as indicated (Panels A to E) were acquired after direct infusion of the derivatized mixture solution at collision energy of 23 eV and collision gas pressure of 1 mTorr as described under "Materials and Methods". Panel A demonstrated that the limit of quantification of 4-HNE after carnosine derivatization was at much lower than 560 amol/μL. The constant intensity ratios of 4-HNE *vs*. d_3-4-HNE ions (Panel F) determined with different concentrations of the mixtures indicate a broad linear dynamic range of quantification after carnosine derivatization.

Figure S2

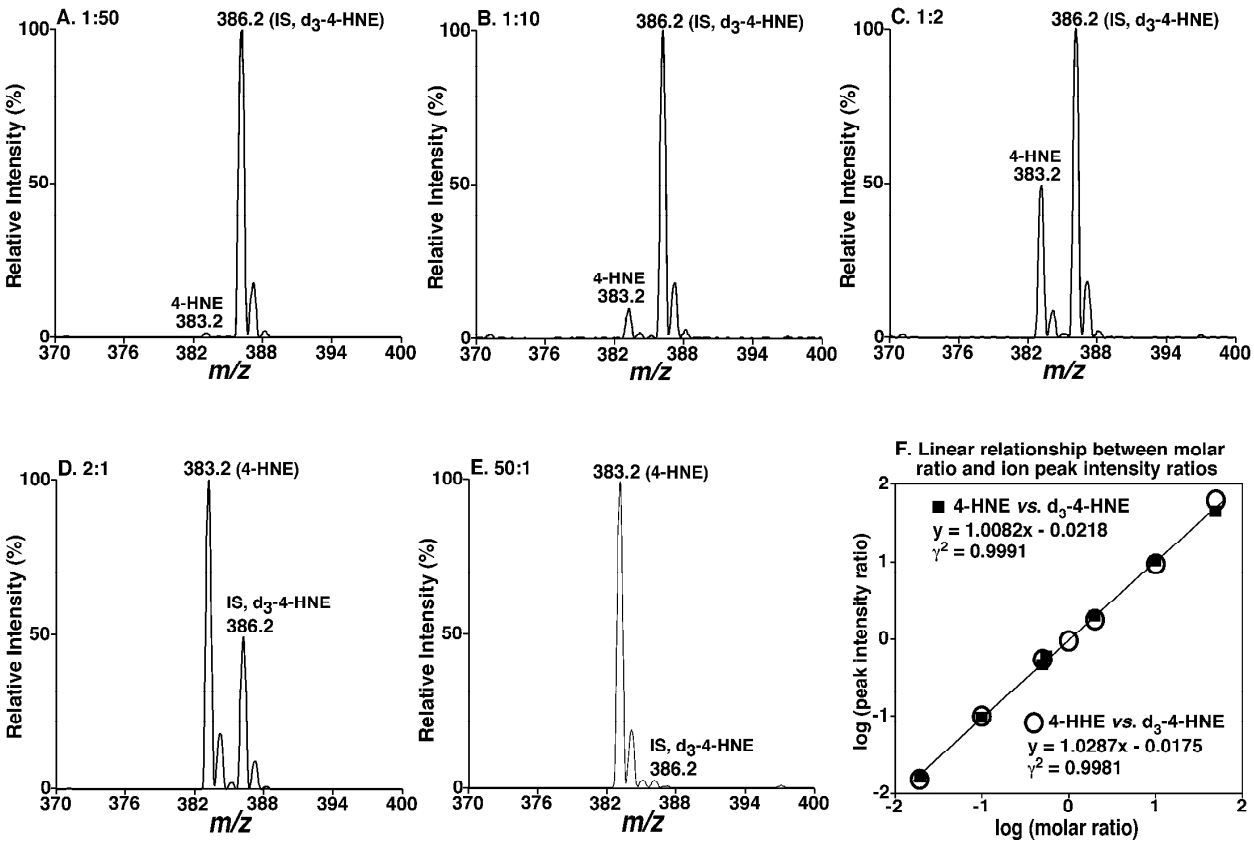

Figure S2. Linear correlation of the molar ratios with the ion peak intensity ratios between 4-hydroxyalkenal species and d_3-4-HNE. Mixtures composed of different molar ratios of 4-HNE *vs*. d_3-4-HNE or 4-HHE *vs*. d_3-4-HNE, in which the concentration of d_3-4-HNE was fixed at 100 fmol/μl, were prepared separately. Ion peak intensity ratios of these mixtures were determined by neutral loss MS analysis of 71.0 and 117.0 Da as described similarly in the legends of Figure 2 and representative neutral loss mass spectra of 71.0 Da at different molar ratios of 4-HNE vs. d_3-4-HNE are displayed in Panels A to E. Linear relationship of the molar ratios *vs*. the ion peak intensity ratios between 4-HNE and d_3-4-HNE (Solid square in Panel F) or 4-HHE and d_3-4-HNE (Open circle in Panel F) was determined after least squares regression.

Figure S3

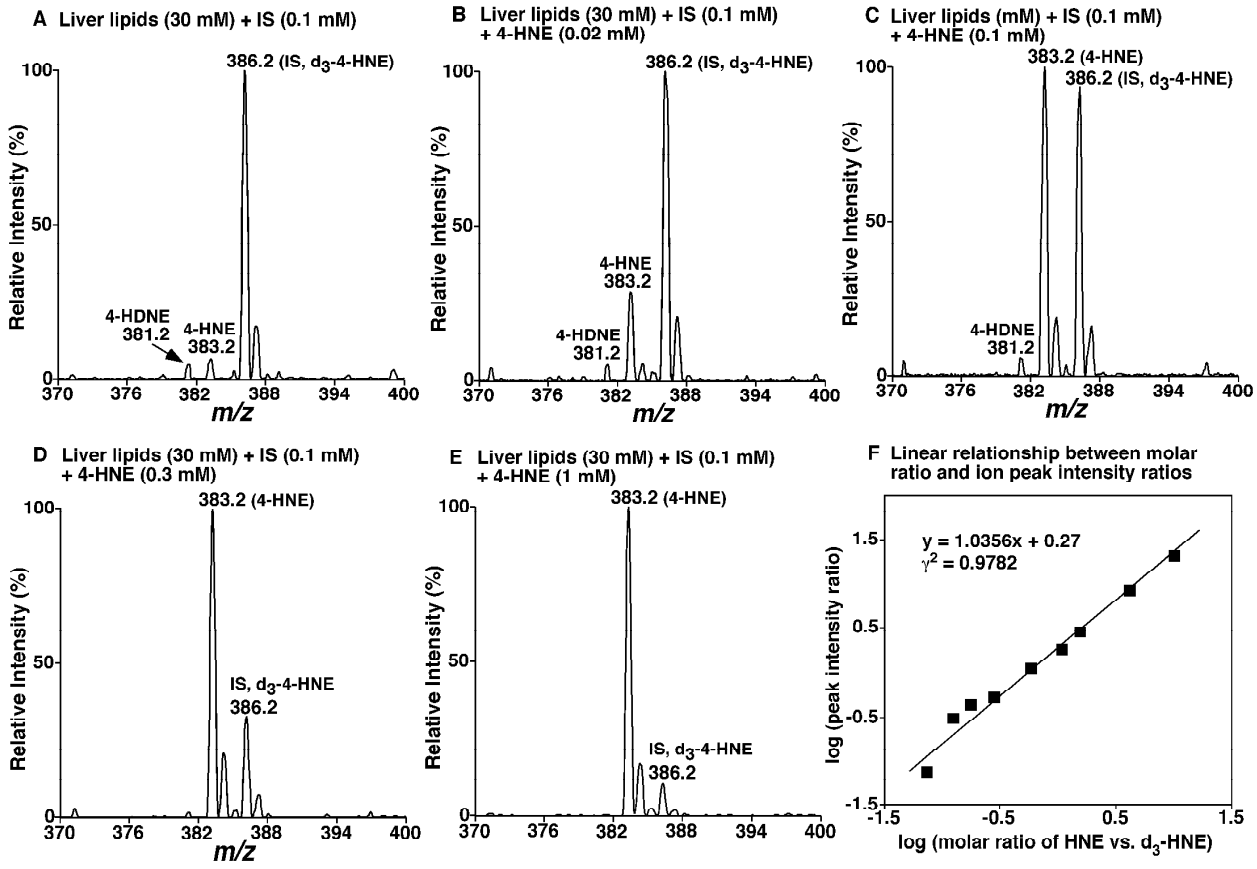

Figure S3. The effects of other lipids present in the matrix on quantification of 4-hydroxyalkenal species with d_3-4-HNE used as an internal standard. Mouse liver lipid extract (~ 250 nmol total lipids/mg protein) was used as a matrix for 4-HNE measurement in which 4-HNE content was determined as 0.40 ± 0.03 nmol/mg protein) (see Table 1). In a liver lipid solution (~ 250 pmol/μl), d_3-4-HNE was spiked as a fixed concentration of 20 pmol/μl whereas 4-HNE was added by varying from 1.0 to 300 pmol/μl. The prepared solutions were diluted to a total lipid concentration of < 50 pmol/μl prior to direct infusion to the mass spectrometer with a nanomate device. The ion peak intensity ratios of 4-HNE and d_3-4-HNE present in the lipid solutions were determined by neutral loss mass spectrometric analyses of 71.0 (Panel A to E) and 117.0 Da (spectra not shown) as described similarly in the legends of Figure 2. Linear relationship of the molar ratios *vs*. the ion peak intensity ratios between 4-HNE and d_3-4-HNE was determined after least squares regression (Panel F).