Supplementary Material

Insights into stoichiometry of arginine modification by phenylglyoxal and 1,2-cyclohexanedione probed by LC-ESI-MS

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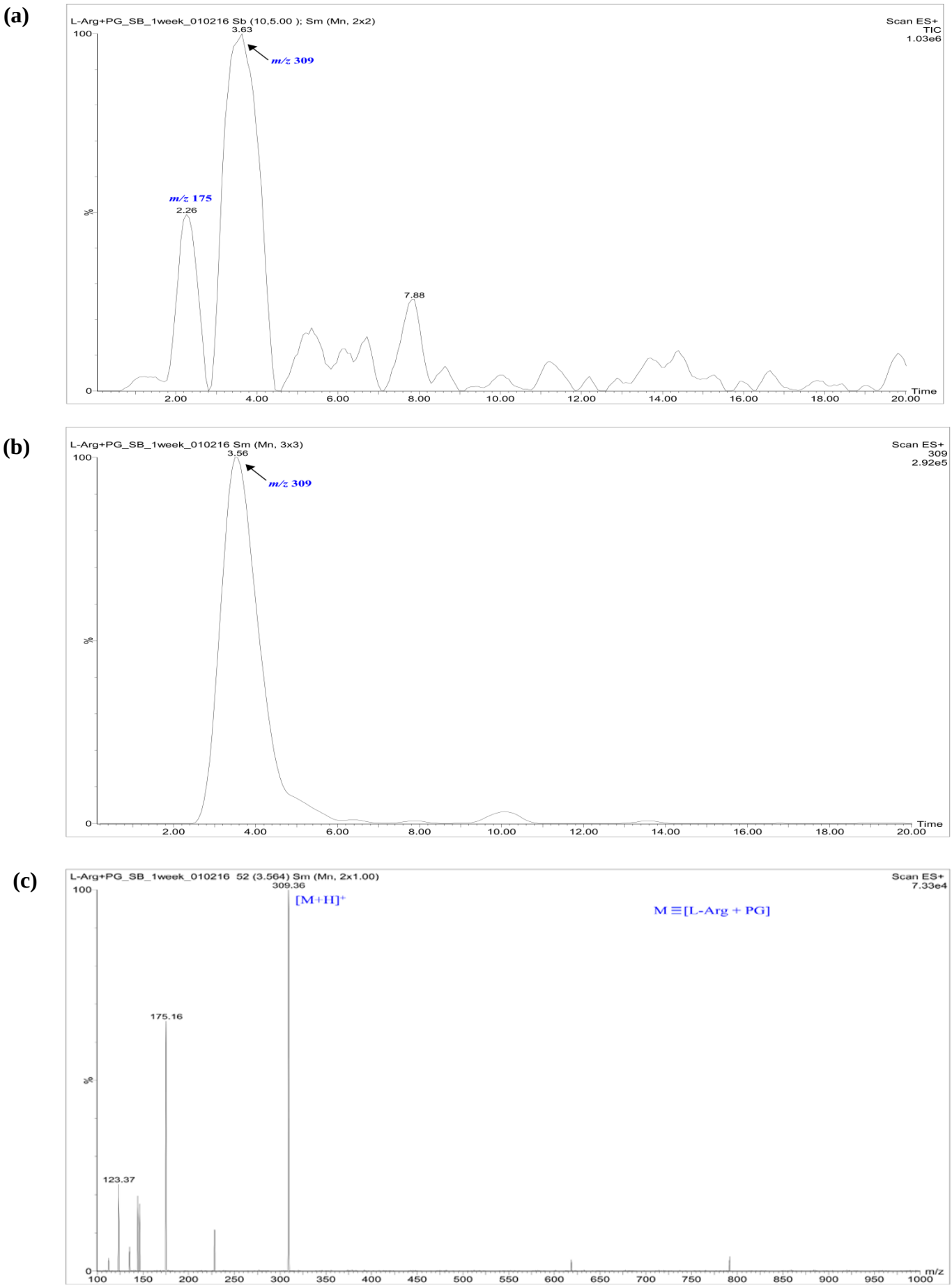


Figure S1. LC-ESI-MS of PG-modified L-Arg (equimolar, working conc. ~ 5 µmole), recorded after about one week: **(a)** Total Ion Chromatogram; **(b)** Extracted Ion chromatogram for *m/z* 309; **(c)** Mass Spectrum at t_R: 3.56 min. This indicates better stability of the product at *m/z* 309 in borate buffer.

Figure S2. Acetylation of PG-modified L-Arg in borate (buffer **1**): **(a)** The peak at *m/z* 351.26, in LC-ESI mass spectrum is indicative of acetylated product; **(b)** LC-ESI-MS/MS spectrum of precursor ion *m/z* 351, acquired at collision energy 30 eV.

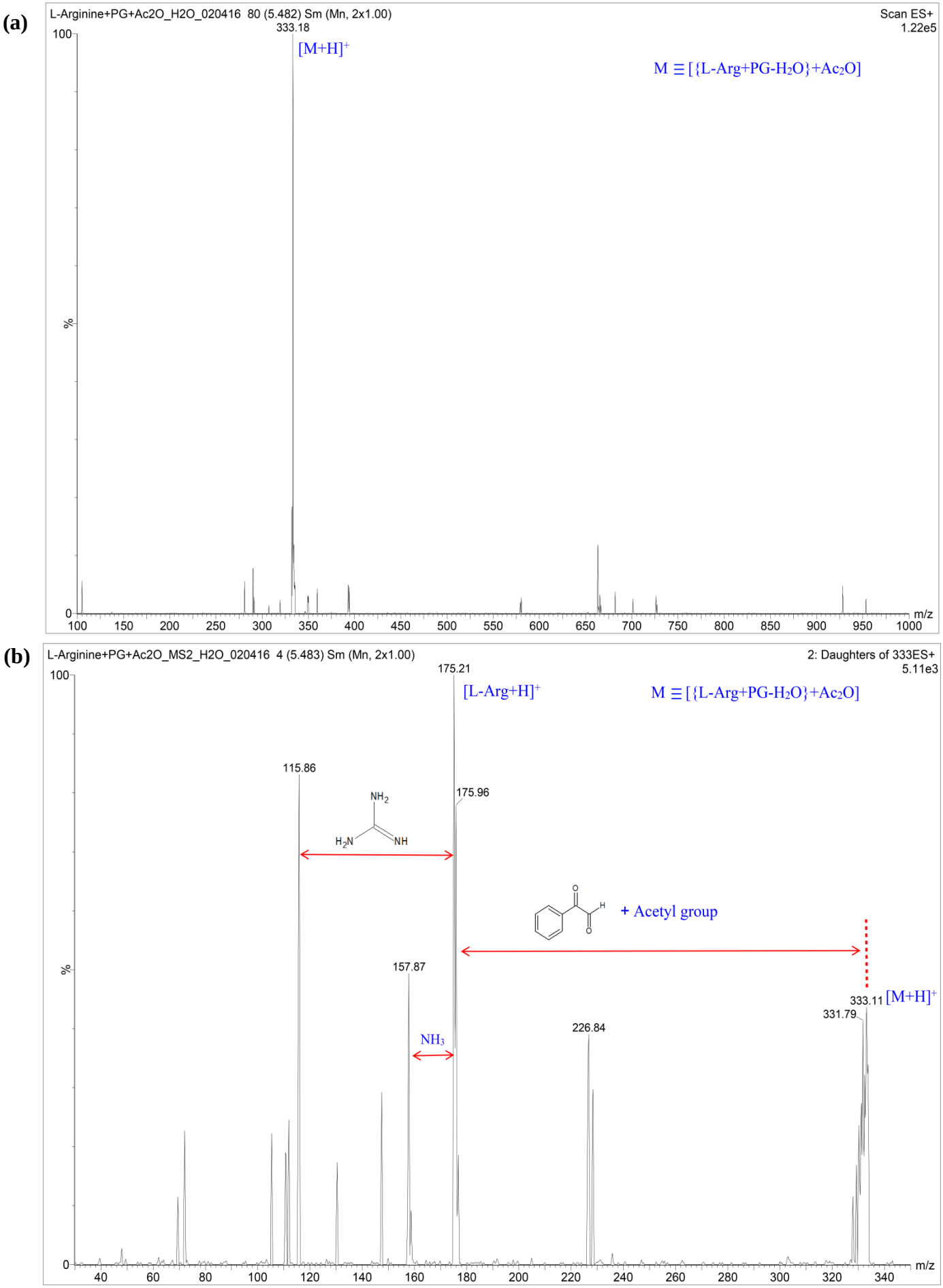


Figure S3. Acetylation of PG-modified L-Arg in H_2O (solvent **6**): **(a)** The peak at *m/z* 333.18 in LC-ESI mass spectrum is suggestive of acetylated product; **(b)** LC-ESI-MS/MS spectrum of precursor ion *m/z* 333, acquired at collision energy 30 eV

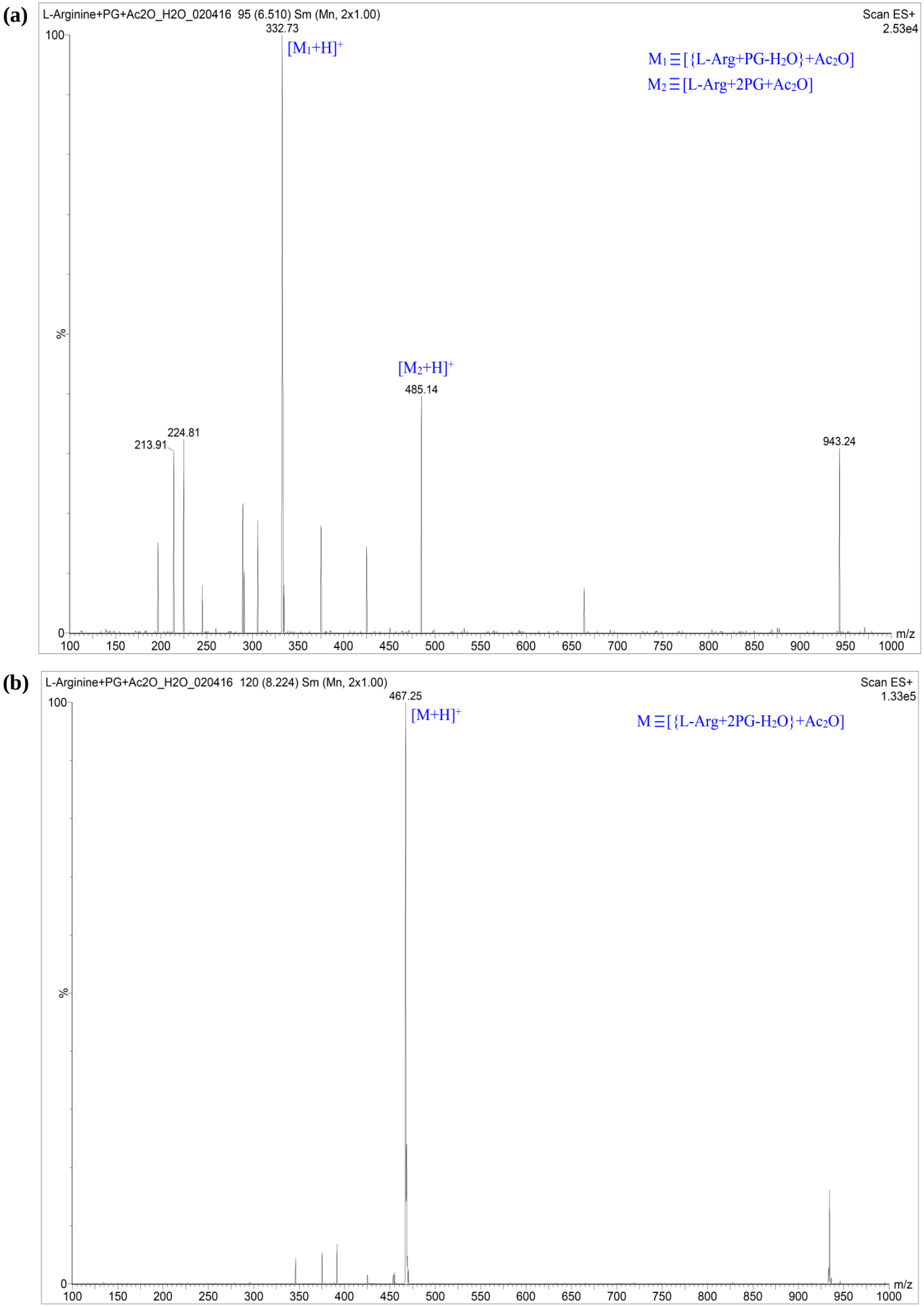
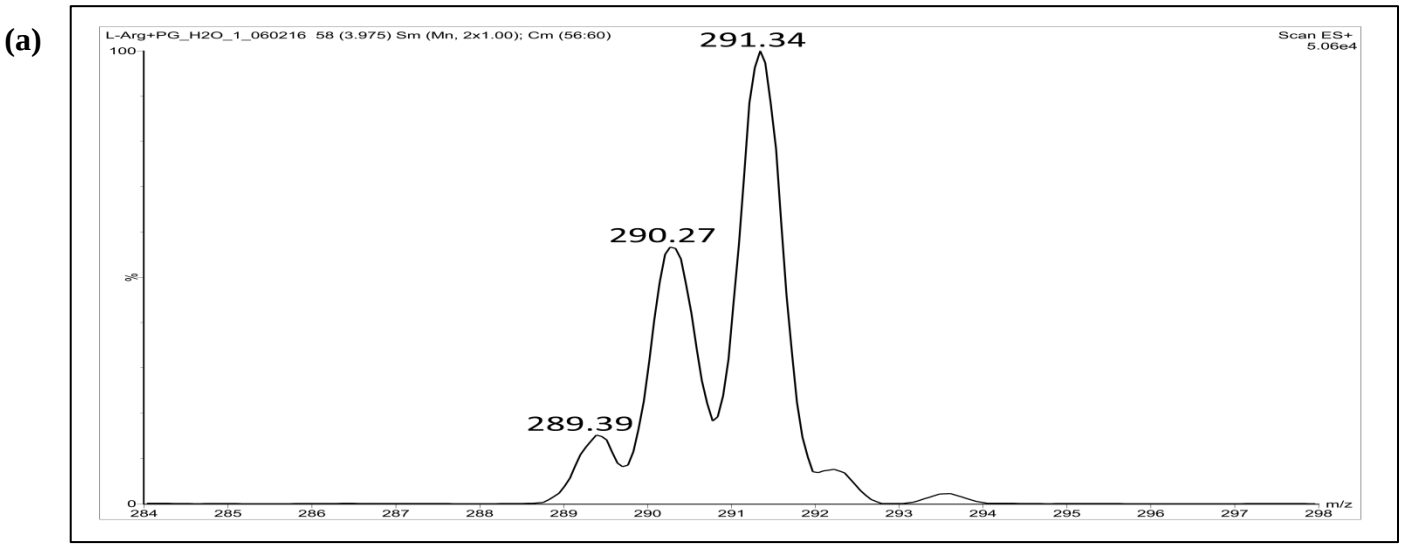
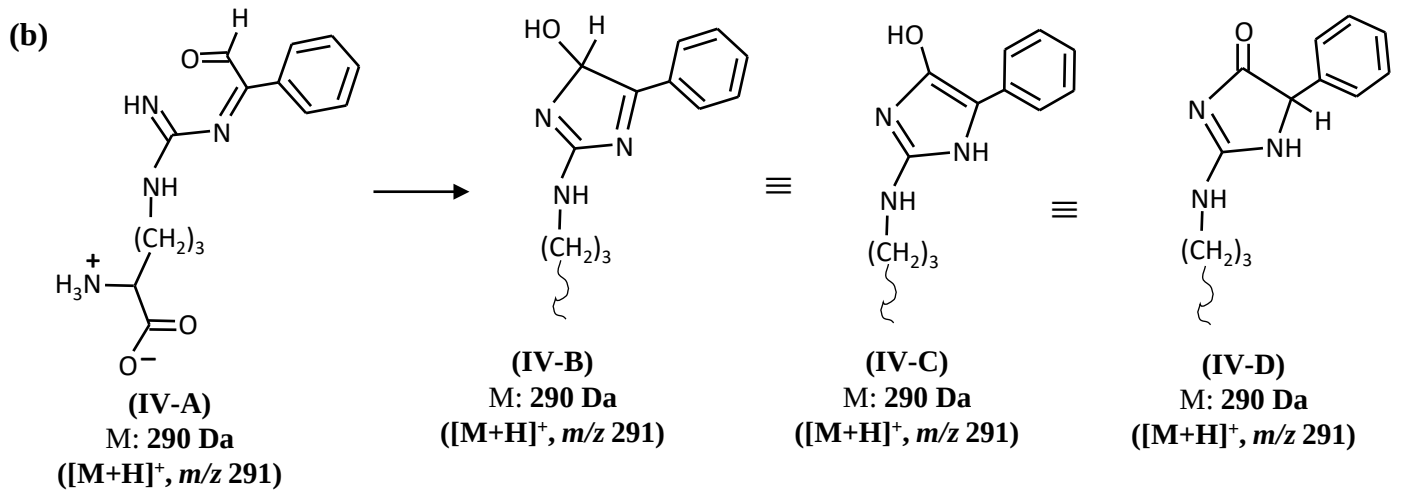
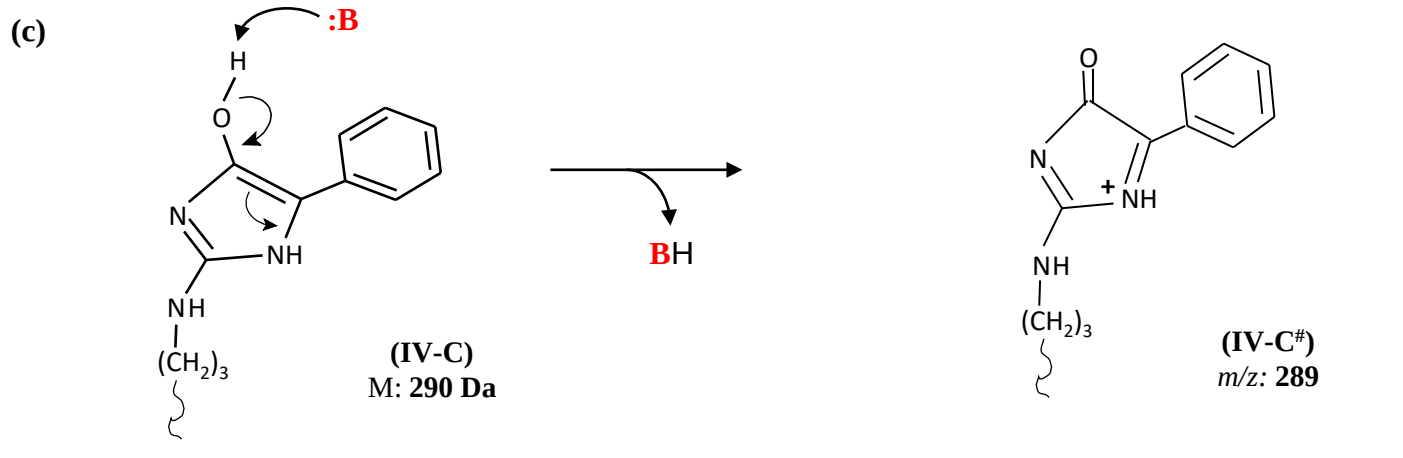


Figure S4. LC-ESI mass spectrum of **(a)** Acetylated [L-Arg+2PG], *m/z* 485.14; **(b)** Acetylated [L-Arg+2PG-H_2O], *m/z* 467.25: detected in H_2O (solvent **6**). Same products were observed from the reaction samples performed in buffers **2 & 3**.







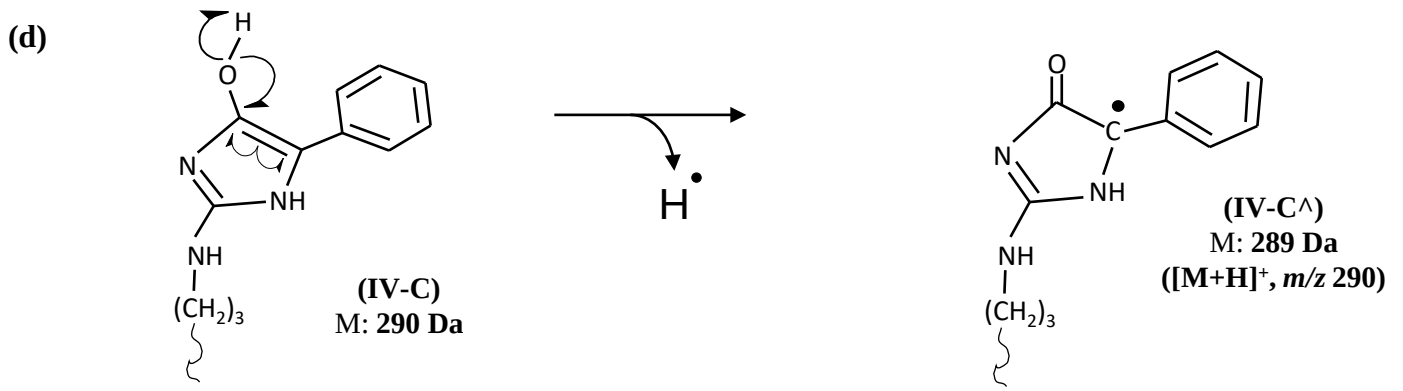
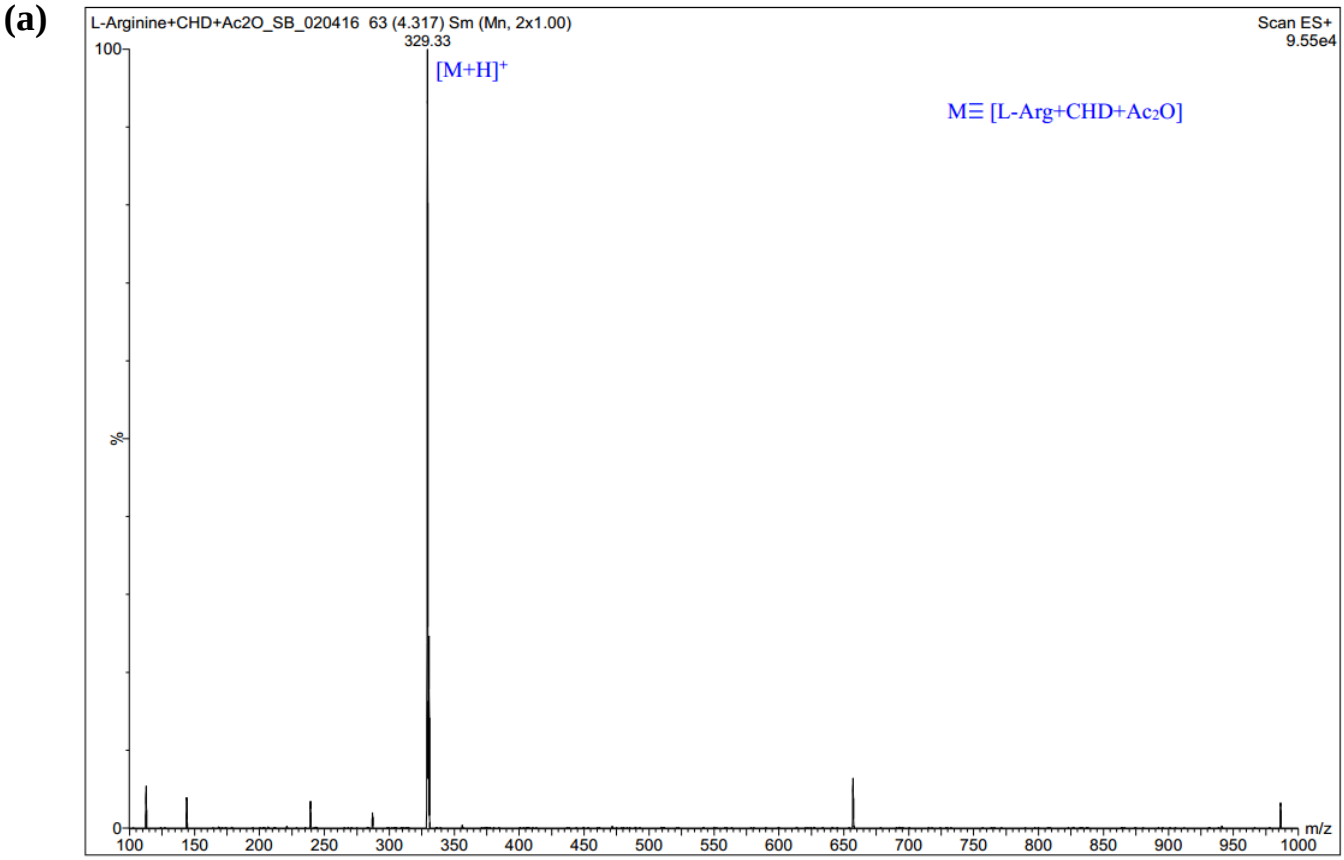


Figure S5. (a) LC-ESI mass spectrum of PG-modified L-Arg in H_2O (solvent **6**). The molecular structures and the mechanisms illustrated in **(b), (c)** and **(d)** may provide rationale for the consistent detection of peaks at *m/z* 289 and *m/z* 290, from the buffers **2** - **5** and solvents **6** & **7**.



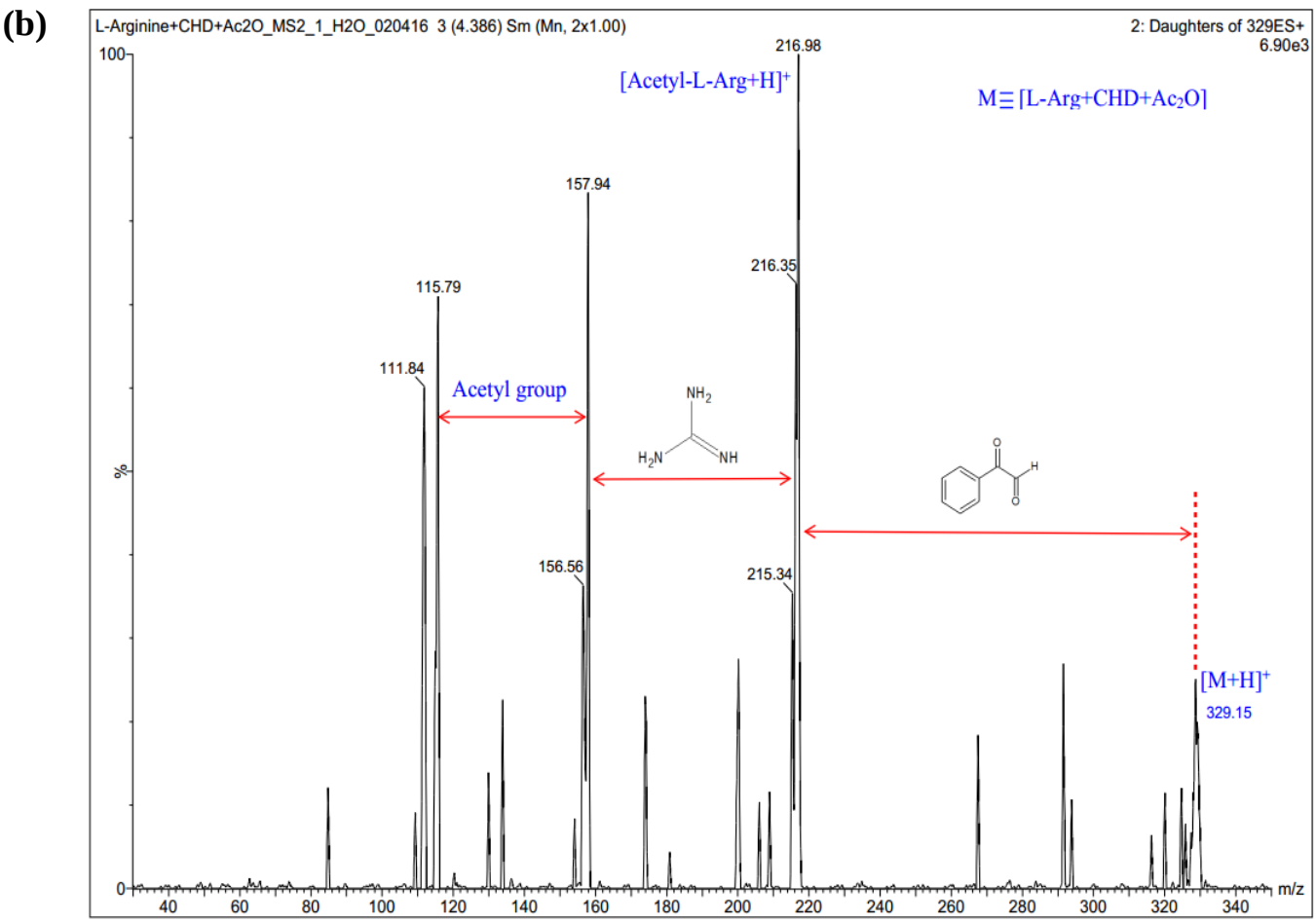


Figure S6. Acetylation of CHD- modified L-Arg in borate (buffer **1**): **(a)** The peak at *m/z* 329.33 in LC-ESI mass spectrum is illustrative of acetylated product; **(b)** LC-ESI-MS/MS spectrum of precursor ion *m/z* 329, recorded at collision energy 35 eV.

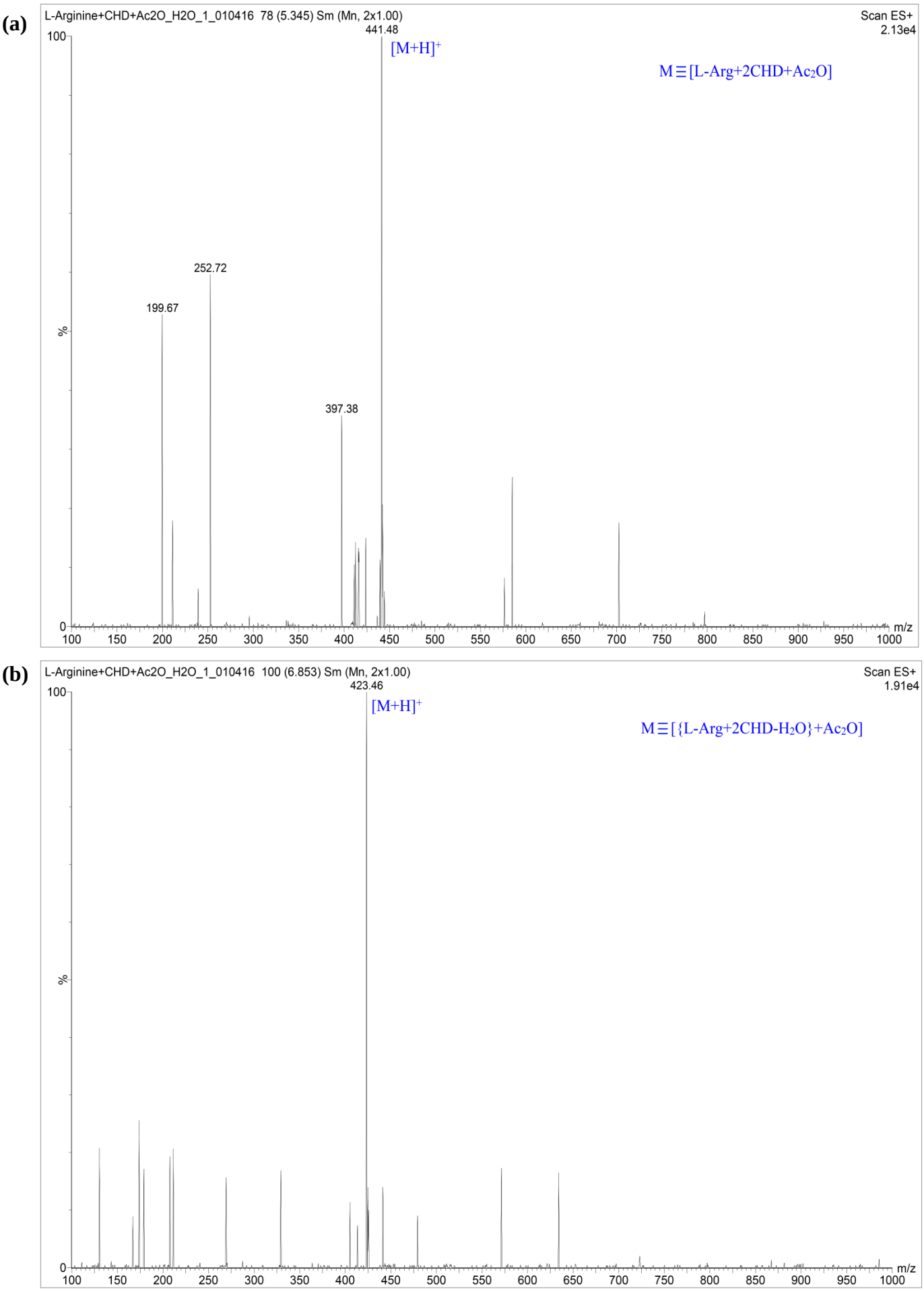


Figure S7. LC-ESI mass spectrum of **(a)** Acetylated [L-Arg+2CHD], *m/z* 441.48; **(b)** Acetylated [L-Arg+2CHD-H_2O], *m/z* 423.46: detected in H_2O (solvent **6**). Same products were observed from the reaction samples performed in buffers **1** & **2**.

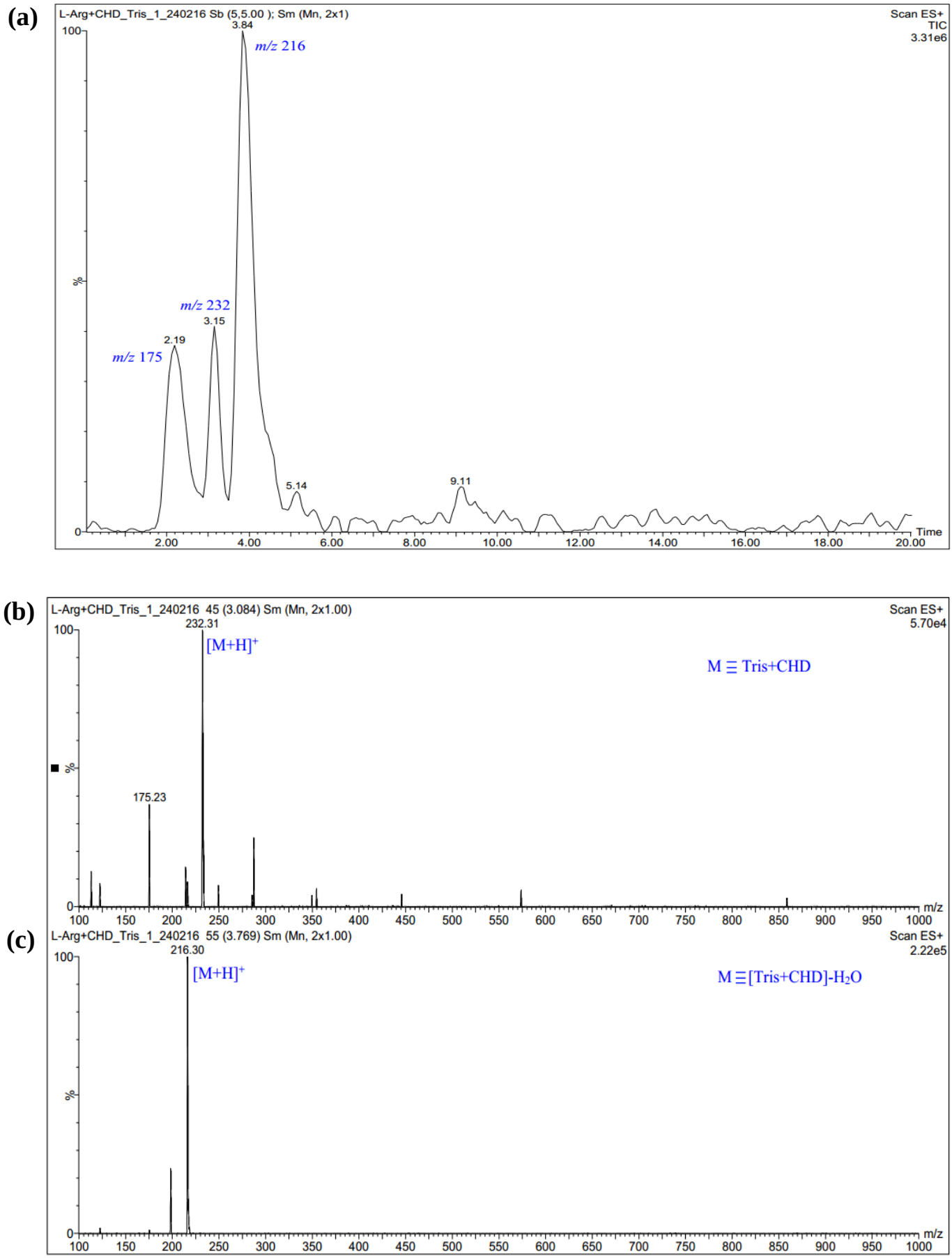


Figure S8. (a) Total Ion Chromatogram (TIC) of [L-Arg + CHD] reaction mixture in Tris-HCl (buffer **3**); Mass spectrum at t_R: 3.08 min **(b)** and at t_R: 3.77 min **(c)**, showing peaks that may be ascribed to the products formed due to reaction between Tris and CHD.

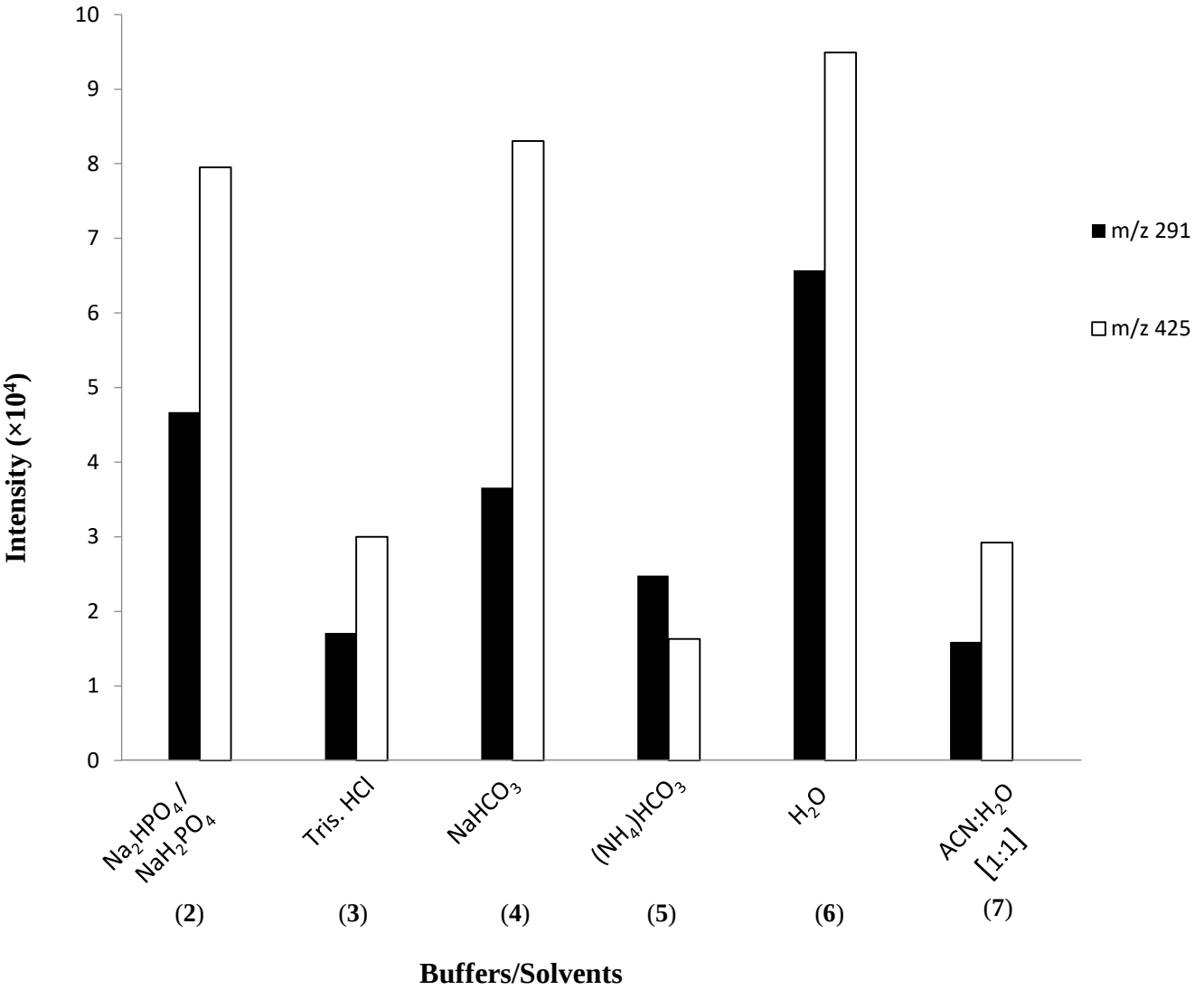


Figure S9. Comparison of populations of 'water condensed 1:1 adduct' (*m/z* 291) and 'water condensed 1:2 adduct' (*m/z* 425) in buffers **2** - **5** and solvents **6** and **7** (equimolar working conc. ~ 5 µmole). In each medium, PG modification of L-Arg was performed three times separately and LC-ESI-MS data were recorded for each of those three reaction mixture. Peak intensities of *m/z* 291 and *m/z* 425 noted after 1 hour from each of those three LC-ESI-MS data were averaged to plot bar graph for every medium.