# Supplementary Materials: A Mediterranean *Alexandrium taylorii* (Dinophyceae) Strain Produces Goniodomin A and Lytic Compounds but Not Paralytic Shellfish Toxins

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**Table S1.** Reference LSU and ITS DNA sequences for *A. taylorii*.

| Strain | Geographic origin | Reference | Gene | Genbank Accession no. |
|---|---|---|---|---|
| AY10T | Adriatic Sea, Lagoon of Marano, Italy | Penna et al. [1] | ITS | AM296013.1 |
| AY2T | Adriatic Sea, Lagoon of Marano, Italy | John et al. [2] | LSU | AJ535348 |
| AY4T | Adriatic Sea, Lagoon of Marano, Italy | John et al. [2] | LSU | AJ535349 |
| AY7T | Adriatic Sea, Lagoon of Marano, Italy | Penna et al. [1] | ITS | AM296012.1 |
| AY1T | Adriatic Sea, Lagoon of Marano, Italy | John et al. [2], Penna et al. [1] | LSU<br>ITS | AJ535347<br>AM296011.1 |
| CBA-1 | Aegean Sea, Kavala, Greece | Penna et al. (unpublished) | ITS | AJ416856.1 |
| AT4 | Ionian Sea, Siracusa, Italy | Penna [3] | ITS | AJ251653.1 |
| B2 | Tyrrhenian Sea, Vulcano, Italy | Penna et al. (unpublished) | ITS | AJ300451.1 |
| AV-8 | Catalan Sea, La Fosca, Spain | Penna [3] | ITS | AJ251654.1 |
| Field sample | Adriatic Sea, Cesenatico, Italy | Penna et al. (unpublished) | ITS | AM296010.1 |
| Temporary-cyst | Tyrrhenian Sea, Vulcano, Italy | Penna et al. (unpublished) | ITS | AJ291785 |
| VGOE6 | Catalan Sea, La Fosca, Spain | Penna et al. [1] | ITS | AM236856 |
| VGO705 | Catalan Sea, Paguera, Spain | Penna et al. [1] | ITS | AM296014.1 |
| Atay99Shio-01 | Shioya Bay, Okinawa Pref., Japan | Nagai and Itakura [4] | LSU | AB607263.1 |
| Atay99Shio-02 | Shioya Bay, Okinawa Pref., Japan | Nagai and Itakura [4] | LSU | AB607264.1 |
| Atay99Shio-03 | Shioya Bay, Okinawa Pref., Japan | Nagai and Itakura [4] | LSU | AB607265.1 |
| Atay99Shio-06 | Shioya Bay, Okinawa Pref., Japan | Nagai [5] | ITS[#] | AB841262.1 |
| AY7T | Adriatic Sea, Lagoon of Marano, Italy | **This study** | LSU<br>ITS | MT643180<br>MT644478 |

[#]note that in GenBank this entry has the header "*Alexandrium taylorii* genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, strain: Atay99Shio-06" but in fact contains only 538 bp ITS sequence information.

## References

1. Penna, A.; Fraga, S.; Maso, M.; Giacobbe, M.G.; Bravo, I.; Garcés, E.; Vila, M.; Bertozzini, E.; Andreoni, F.; Luglie, A.; et al. Phylogenetic relationships among the Mediterranean *Alexandrium* (Dinophyceae) species based on sequences of 5.8S gene and Internal Transcript Spacers of the rRNA operon. *Eur. J. Phycol.* **2008**, *43*, 163–178.

2. John, U.; Fensome, R.A.; Medlin, L.K. The application of a molecular clock based on molecular sequences and the fossil record to explain biogeographic distributions within the *Alexandrium tamarense* "species complex" (Dinophyceae). *Mol. Biol. Evol.* **2003**, *20*, 1015–1027.
3. Penna, A. Blooms of *Alexandrium taylori* (Dinophyceae) in the Mediterranean: A preliminary molecular analysis of different isolates. In *Harmful Algal Blooms 2000, Proceedings of the 9TH International Conference on hatrmful Algal Blooms, Hobart, Australia*; Hallegraeff, G.M., Blackburn, S.I., Bolch, C.J.S., Lewis, R.J., Eds.; Unesco: Paris, France, 2001; pp. 218–221.
4. Nagai, S.; Itakura, S. Specific detection of the toxic dinoflagellates *Alexandrium tamarense* and *Alexandrium catenella* from single vegetative cells by a loop-mediated isothermal amplification method. *Mar. Genom.* **2012**, *7*, 43–49.
5. Nagai, S. Species-specific detection of six *Alexandrium* species from single vegetative cells by a loop-mediated isothermal amplification method. *DNA Test.* **2013**, *5*, 33–46.

**Table S2.** MS parameters of PST and GC toxin analysis.

| Parameter | ESI+ | ESI- |
|---|---|---|
| Soft Transmission Mode | enabled | disabled |
| Capillary (kV) | 0.5 | 2.5 |
| Cone (V) | 10 | 10 |
| Source Offset (V) | 30 | 30 |
| Source Temperature (°C) | 150 | 150 |
| Desolvation Temperature (°C) | 600 | 600 |
| Cone Gas Flow (L $h^{-1}$) | 150 | 150 |
| Desolvation Gas Flow (L $h^{-1}$) | 1000 | 1000 |
| Collision Gas Flow (mL $min^{-1}$) | 0.15 | 0.15 |
| Nebuliser Gas (bar) | 7 | 7 |

**Table S3.** MS/MS parameters of lipophilic toxin analysis.

| Parameter | ESI+ |
|---|---|
| Capillary (kV) | 3.0 |
| Cone (V) | 40 |
| Source Offset (V) | 20 |
| Source Temperature (°C) | 150 |
| Desolvation Temperature (°C) | 600 |
| Cone Gas Flow (L $h^{-1}$) | 150 |
| Desolvation Gas Flow (L $h^{-1}$) | 1000 |
| Collision Gas Flow (mL $min^{-1}$) | 0.15 |
| Nebuliser Gas (bar) | 7 |
| Collision energy (eV) | 40 |

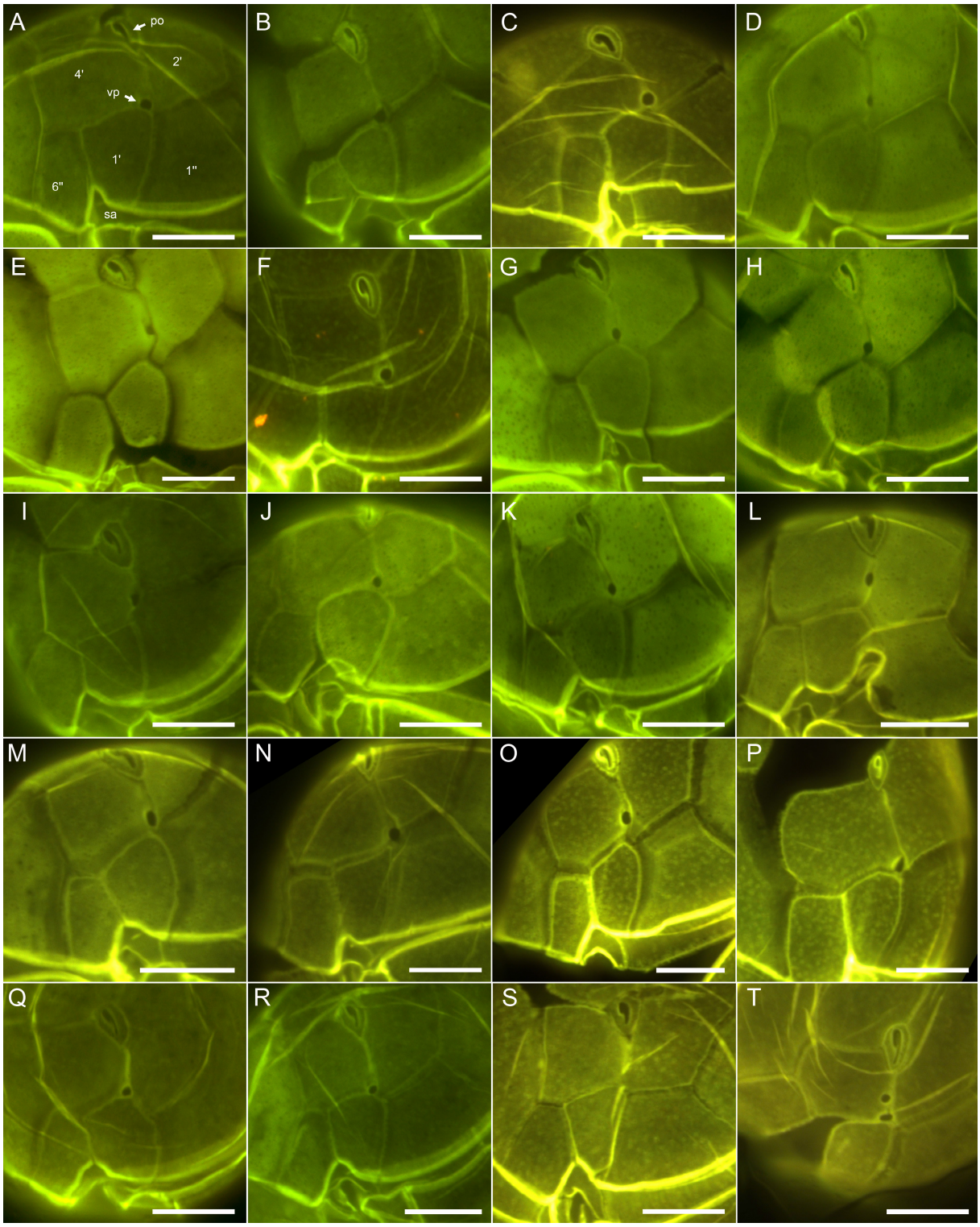


**Figure S1.** *Alexandrium taylorii* AY7T. Detailed ventral views of epithecal plates of different Lugol-fixed cells stained with Solophenyl Flavine and viewed with epifluorescence and blue light excitation to illustrate shape variability of plates 1' and 6" and variability in position of the ventral pore (vp). Note that in (S) no vp could be identified, whereas in (T) two vp were present. Plate label exemplarily shown in (A). Scale bars = 10 µm.

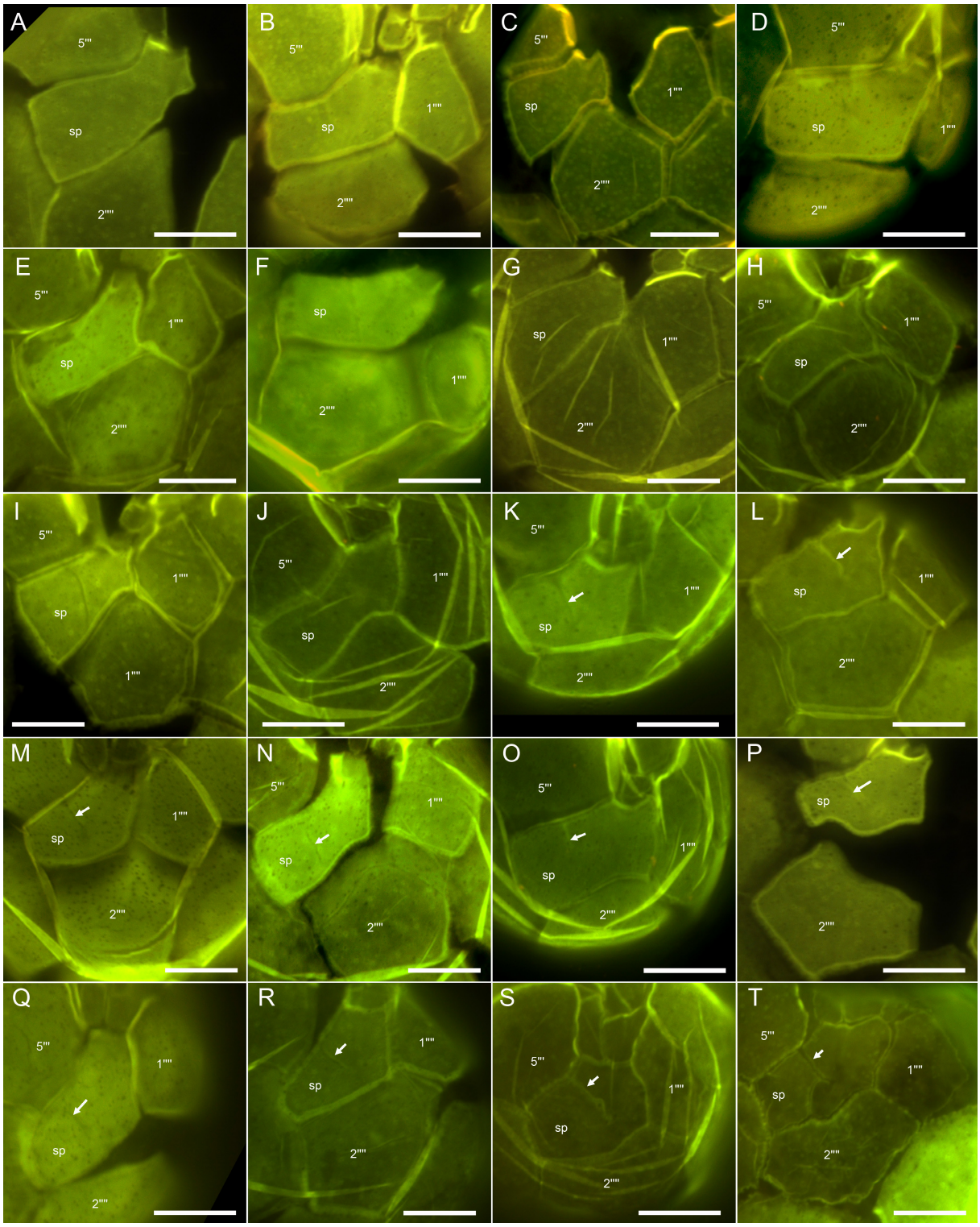


**Figure S2.** *Alexandrium taylorii* AY7T. Detailed ventral or antapical views of hypothecal plates of different Lugol-fixed cells stained with Solophenyl Flavine and viewed with epifluorescence and blue light excitation to illustrate shape variability of the posterior sulcal plate sp. Note the faint groove that extend from the right margin of sp in K–T (arrows) which occasionally ended in a small pore (S) or continued to the left margin (T). Scale bars = 10 μm.

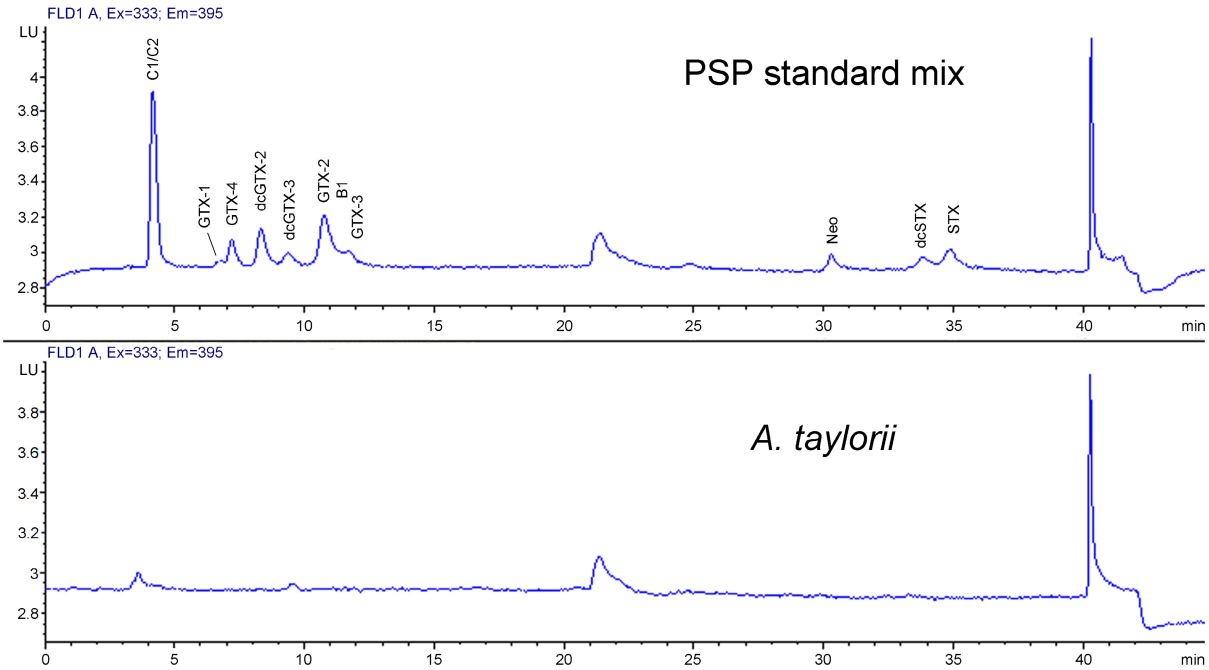


**Figure S3.** LC-FLD chromatograms of a PST standard mix (upper panel) and the *Alexandrium taylorii* extract (lower panel). Concentrations of the PST standard solution are the following: C1: 100.3 pg $\mu L^{-1}$; C2: 28.6 pg $\mu L^{-1}$; GTX1: 205.4 pg $\mu L^{-1}$; GTX4: 54.7 pg $\mu L^{-1}$; dcGTX2: 16.1 pg $\mu L^{-1}$; dcGTX3: 4.5 pg $\mu L^{-1}$; GTX2: 16.3 pg $\mu L^{-1}$; GTX3: 5.4 pg $\mu L^{-1}$; B1: 26.3 pg $\mu L^{-1}$; NEO: 100 pg $\mu L^{-1}$; dcSTX: 12.8 pg $\mu L^{-1}$; STX: 14.7 pg $\mu L^{-1}$.

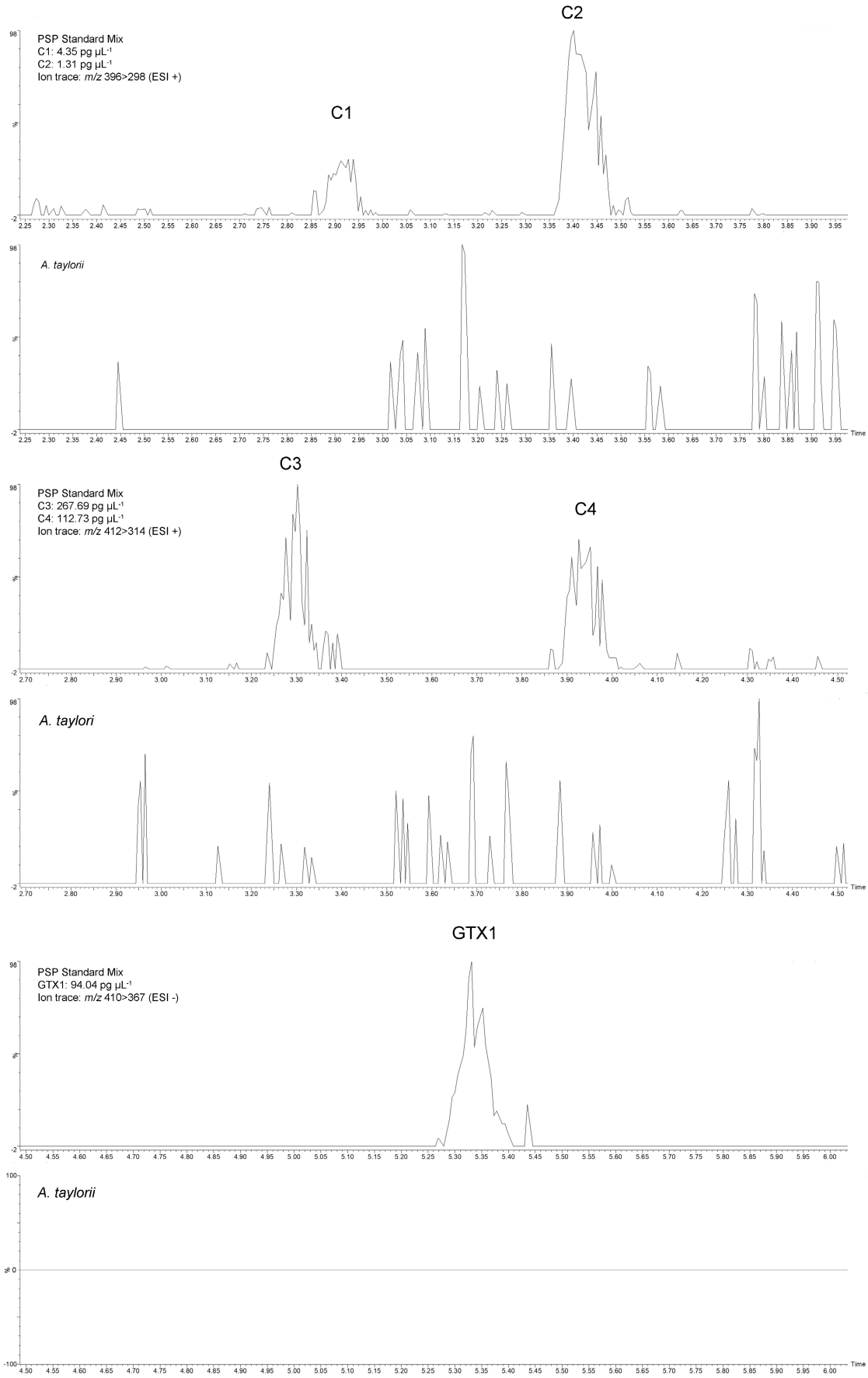


**Figure S4.** Extracted Ion chromatograms of a PST standard mix (upper panels) and the *A. taylorii* extract (lower panels).

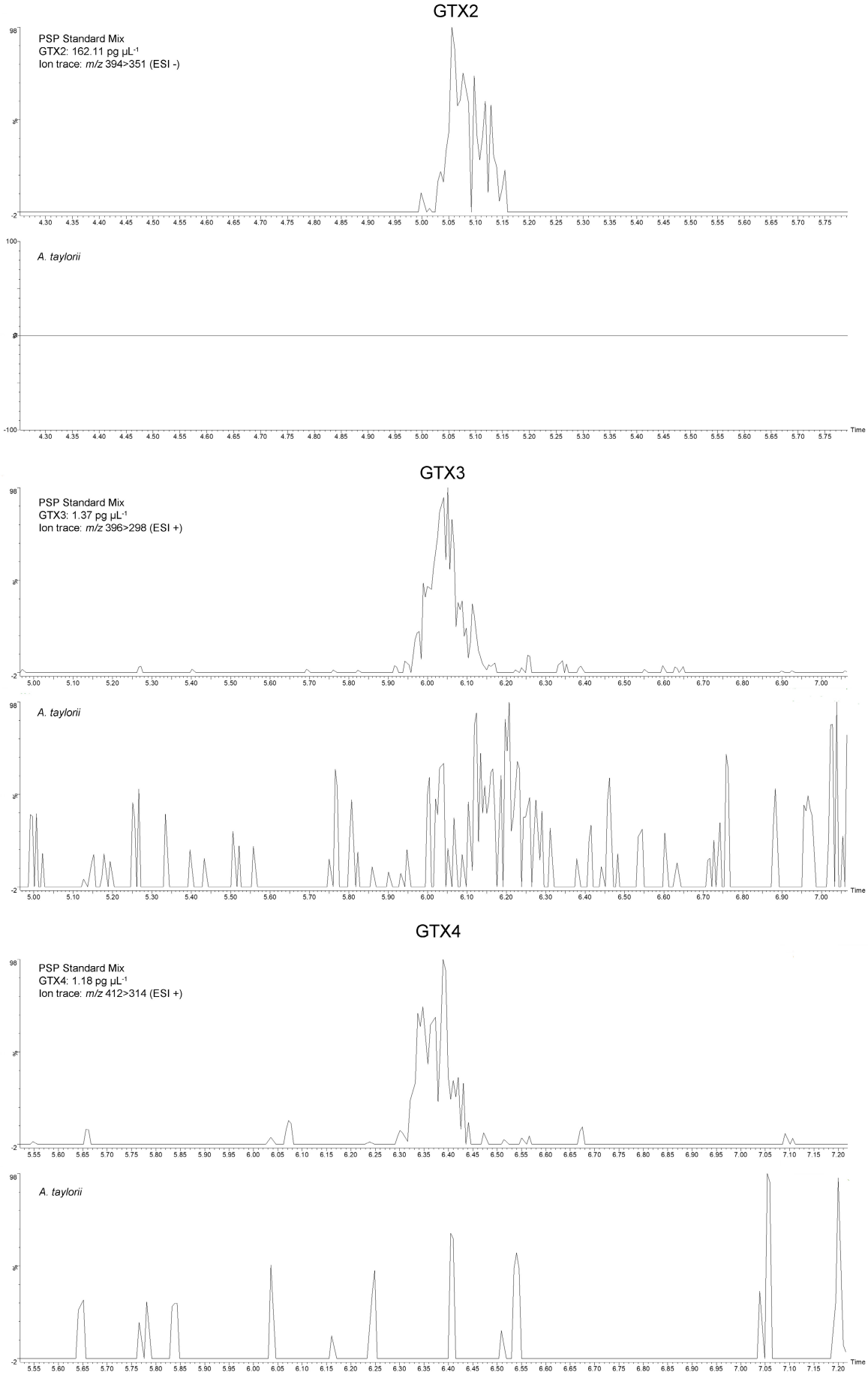


**Figure S4.** continued.

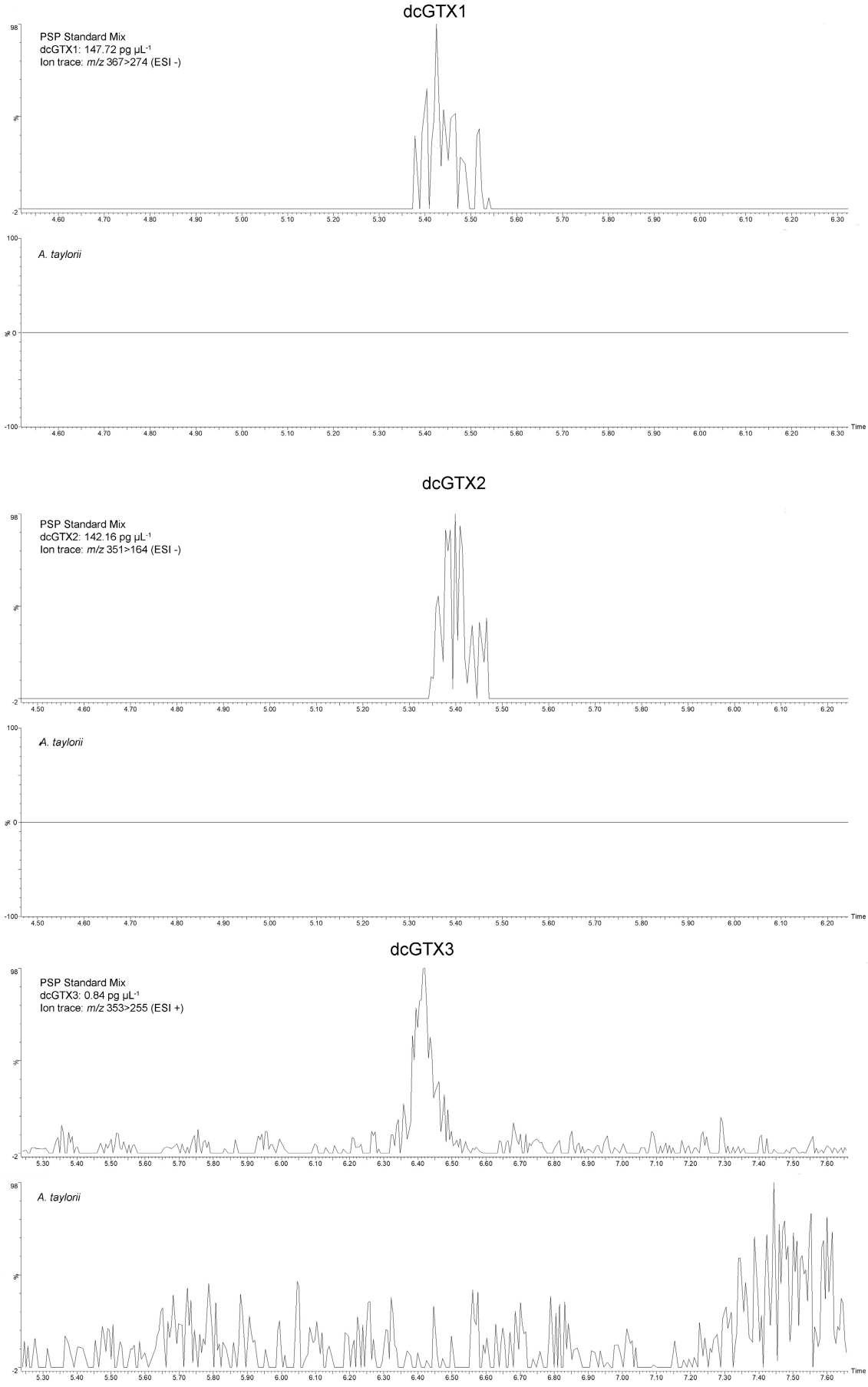


**Figure S4.** continued.

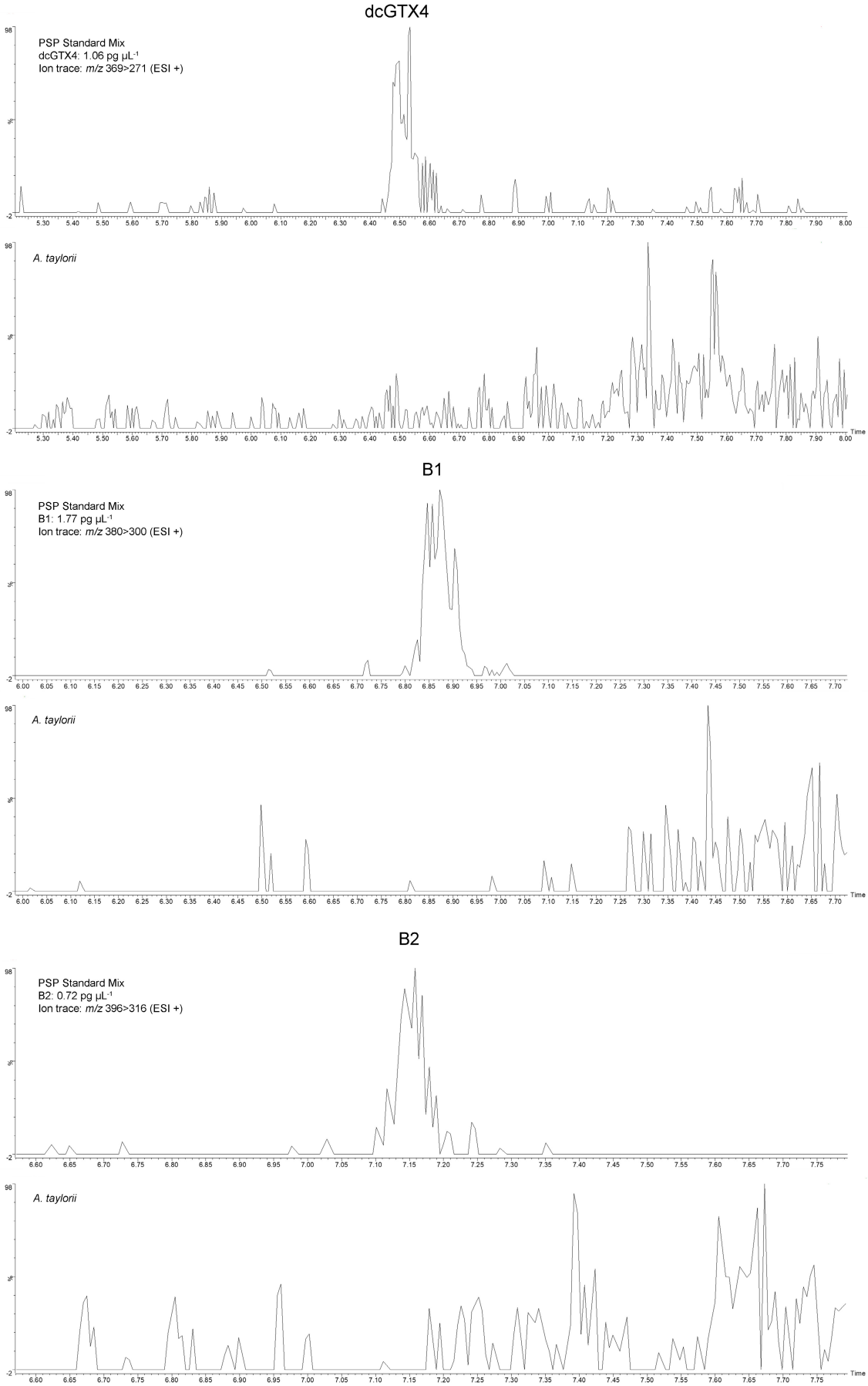


**Figure S4.** continued.

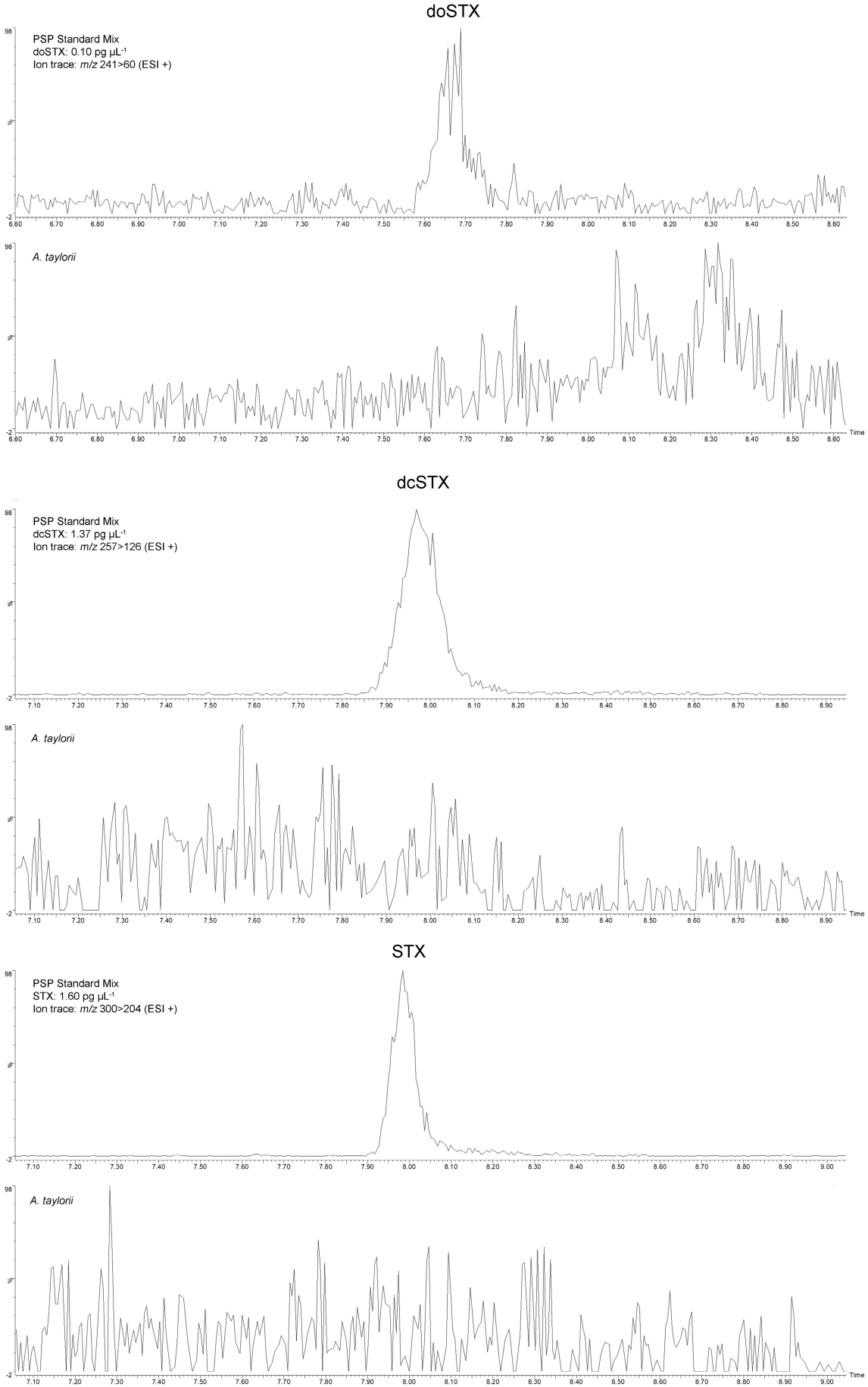


**Figure S4.** continued.

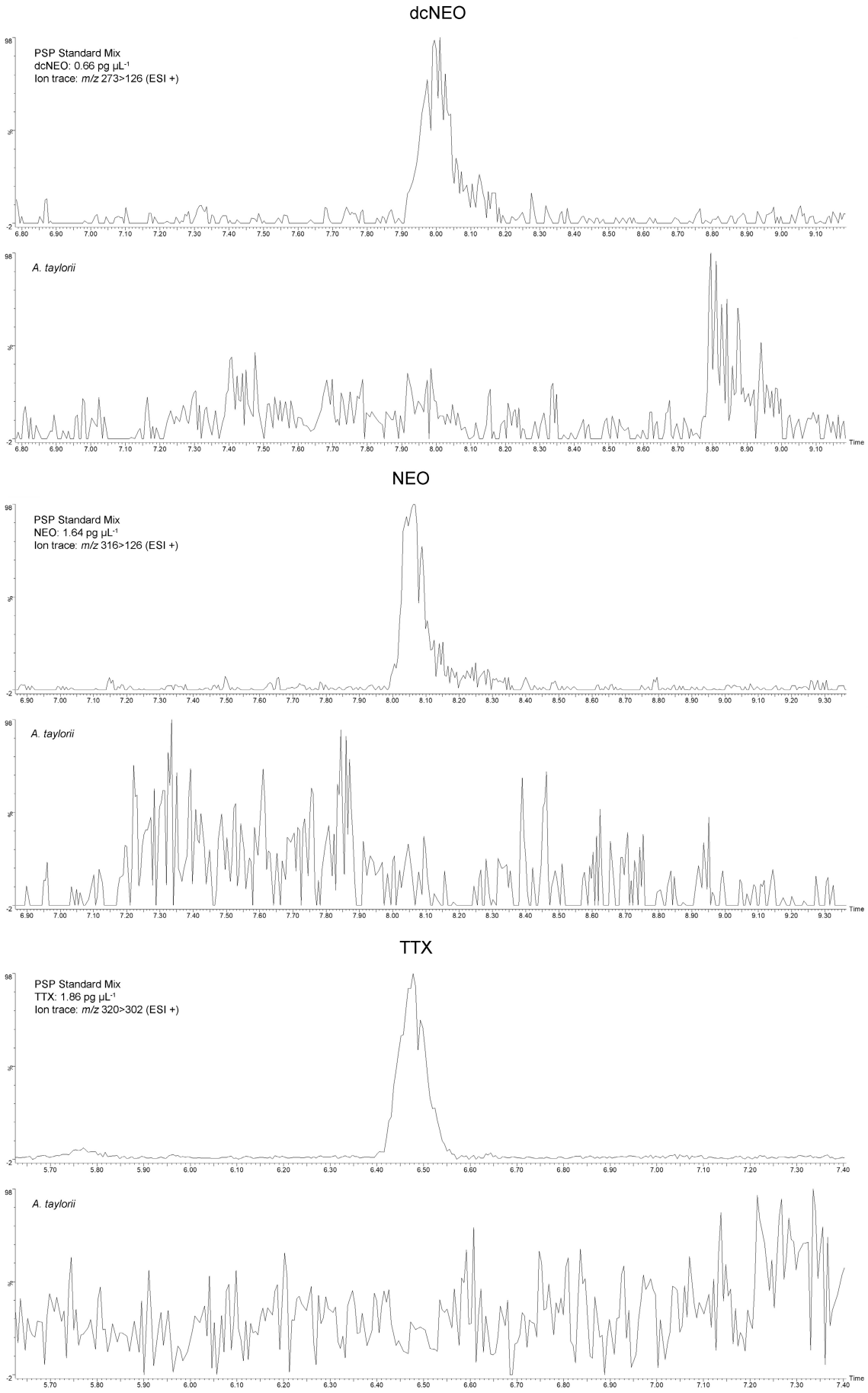


**Figure S4.** continued.