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Article

A Study of the Metabolic Profiles of *Penicillium dimorphosporum* KMM 4689 which Led to Its Re-Identification as *Penicillium hispanicum*

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Abstract: The changes in cultivation conditions, in particular salinity and temperature, affect the production of secondary fungal metabolites. In this work, the extracts of fungus previously described as *Penicillium dimorphosporum* cultivated in various salinity and temperature conditions were investigated by HPLC UV/MS techniques and their DPPH radical scavenging and cytotoxicity ac-tivities against human prostate cancer PC-3 cells and rat cardiomyocytes H9c2 were tested. Totally, 25 compounds, including 13 desoxyisoaustamide-related alkaloids and eight anthraquinones, were identified in the studied extracts and their relative amounts were estimated. The production of known neuroprotective alkaloids **5, 6** and other brevianamide alkaloids was increased in hyper-saline and high temperature conditions and this may be an adaptation to extreme conditions. On the other hand, the hyposalinity stress may induces the synthesis of unidentified antioxidants with low cytotoxicity that can be very interesting for future investigation. The study of secondary metab-olites of the strain KMM 4689 showed that although brevianamide-related alkaloids and anthraqui-none pigments are widely distributed in various fungi, these metabolites have not been described for *P. dimorphosporum* and related species. For this reason, the strain KMM 4689 was re-sequenced using β-tubulin gene and ITS regions as molecular markers and further identified as *P. hispanicum*.

Keywords: *Penicillium dimorphosporum*; *Penicillium hispanicum*; OSMAC; HPLC MS; metabolite profile; ITS; β-tubulin; phylogeny; re-identification; bioactivity; cultivation conditions; salinity; second-ary metabolites; PCA

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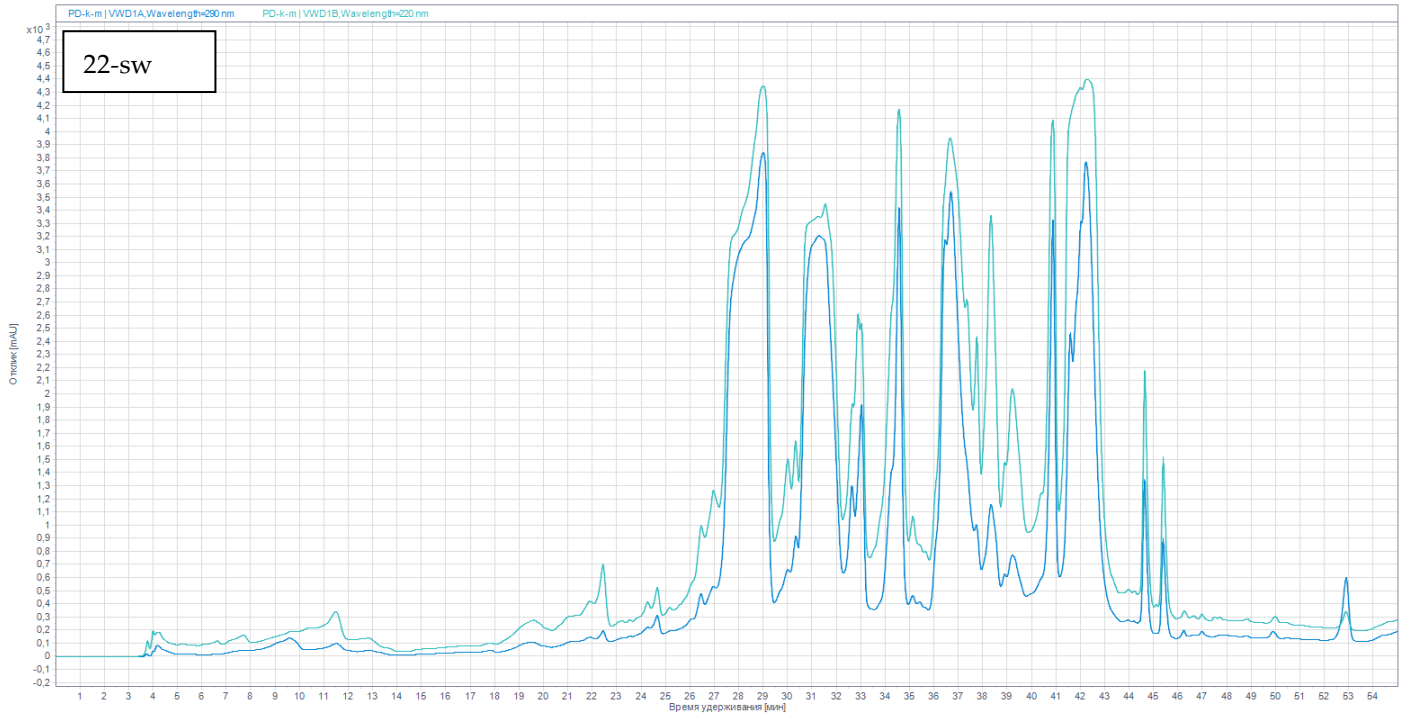

Figure S1. HPLC UV chromatogram of extract from fungus cultivated with sea water at 22 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

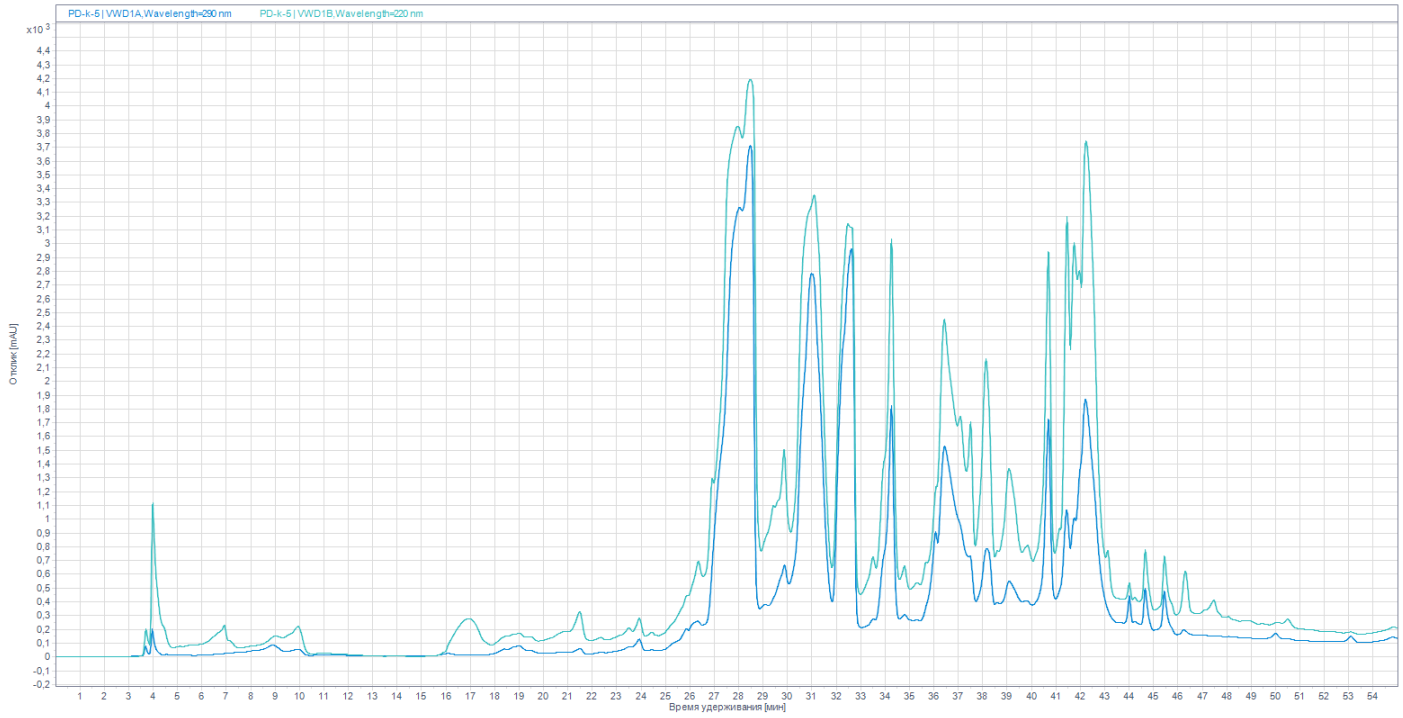

Figure S2. HPLC UV chromatogram of extract from fungus cultivated with 5 g/L sea salt concentration at 22 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

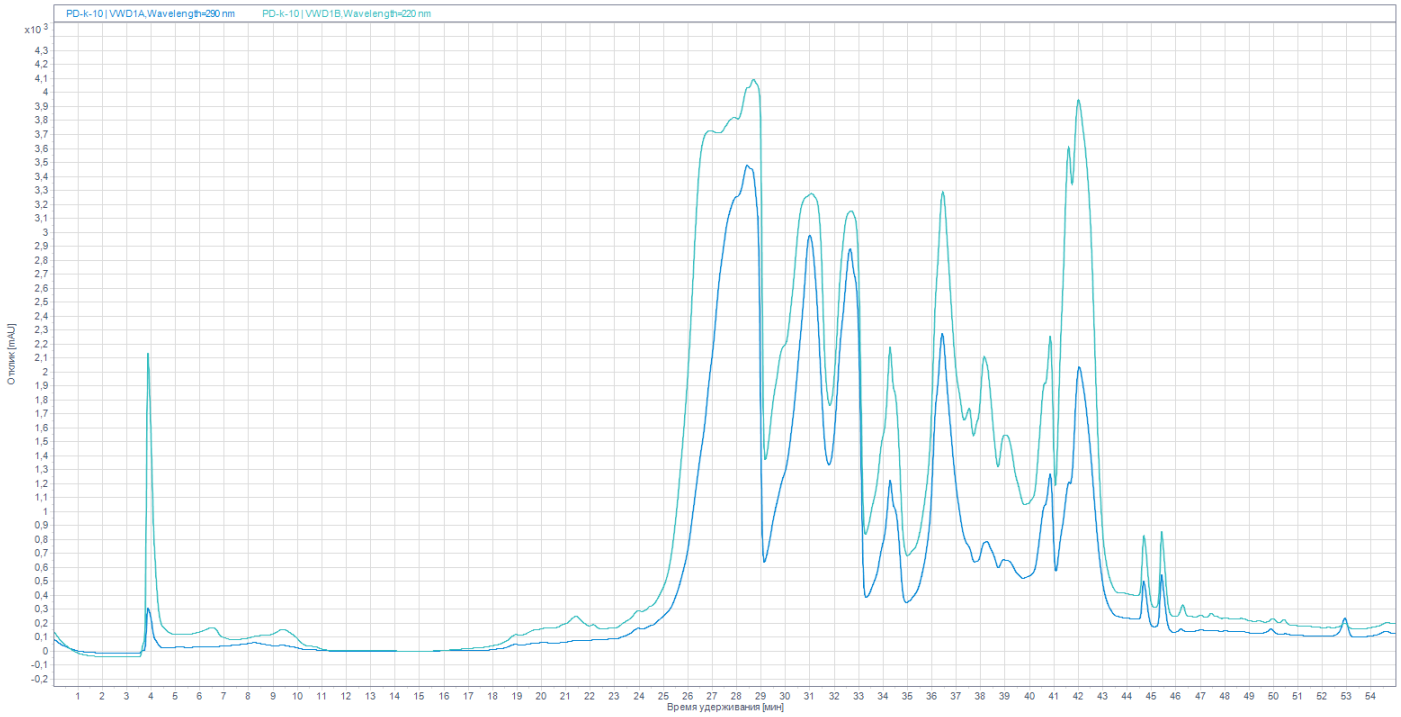

Figure S3. HPLC UV chromatogram of extract from fungus cultivated with 10 g/L sea salt concentration at 22 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

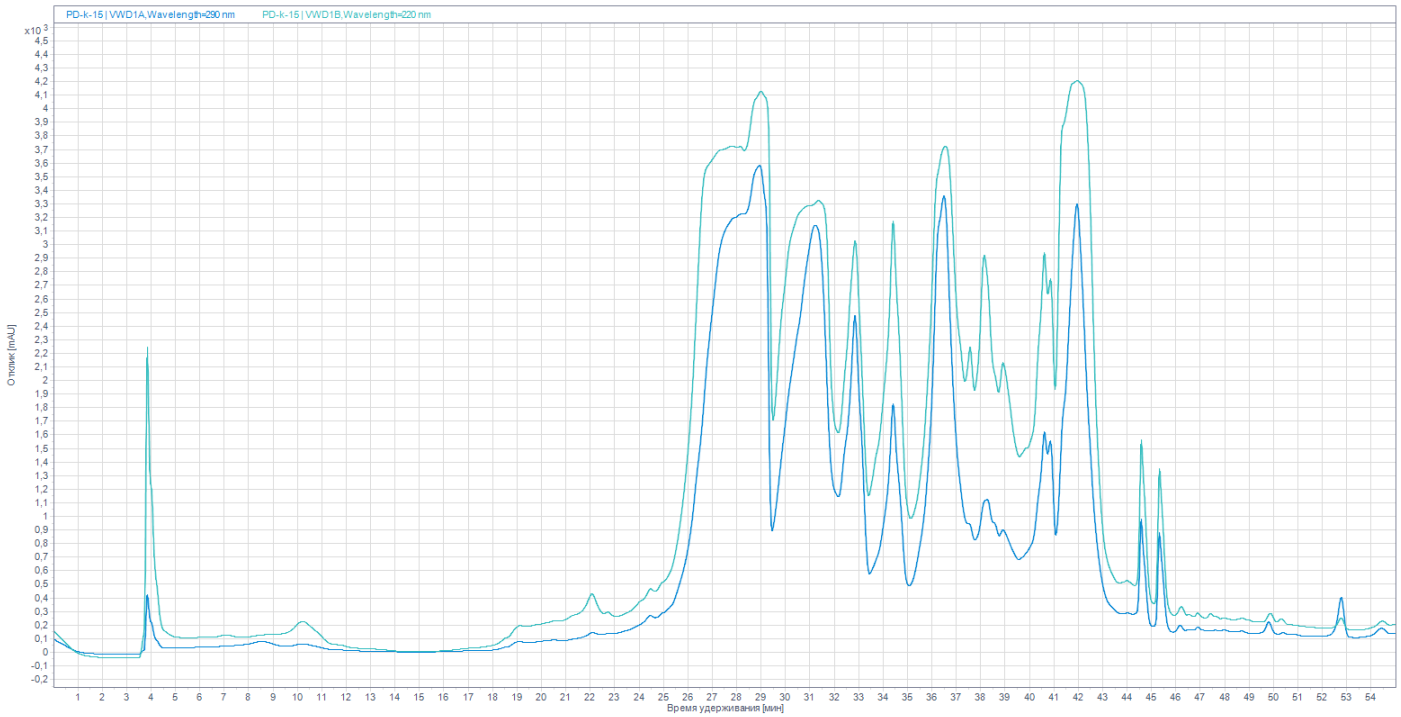

Figure S4. HPLC UV chromatogram of extract from fungus cultivated with 15 g/L sea salt concentration at 22 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

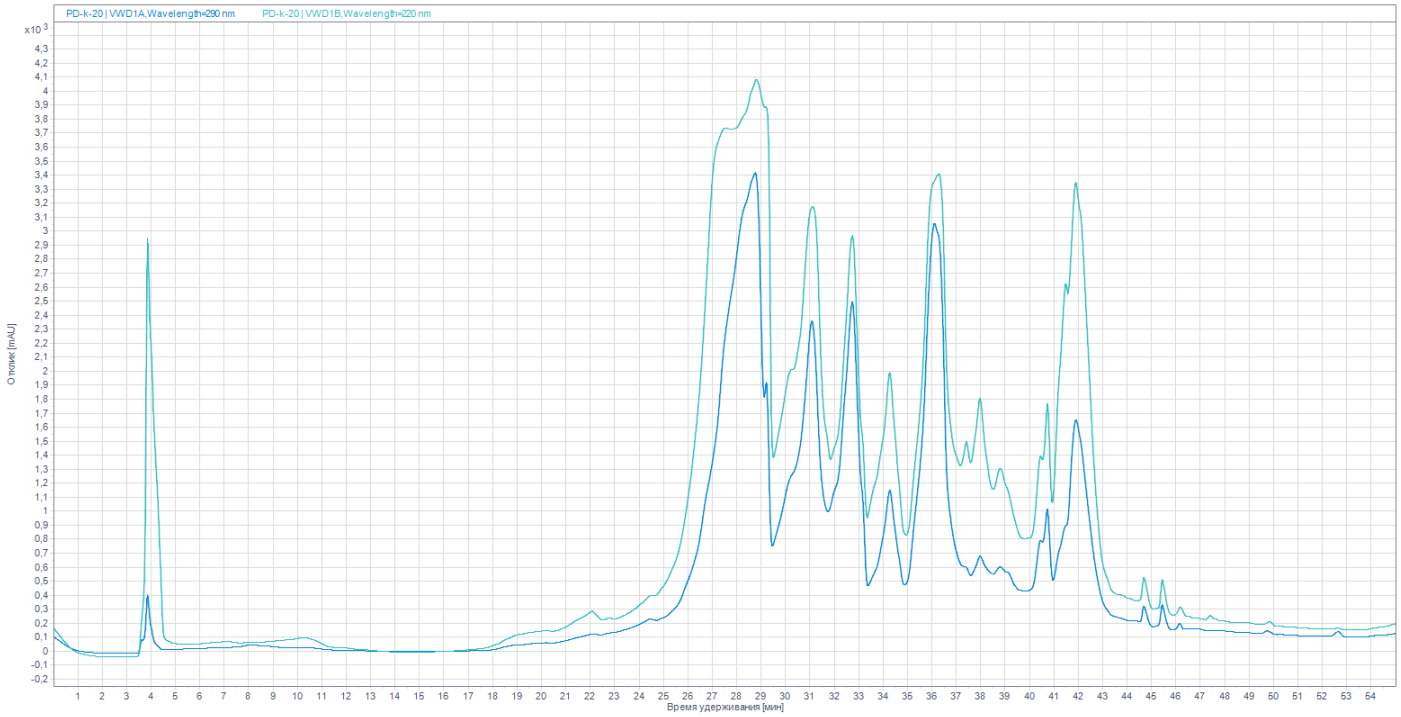

Figure S5. HPLC UV chromatogram of extract from fungus cultivated with 20 g/L sea salt concentration at 22 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

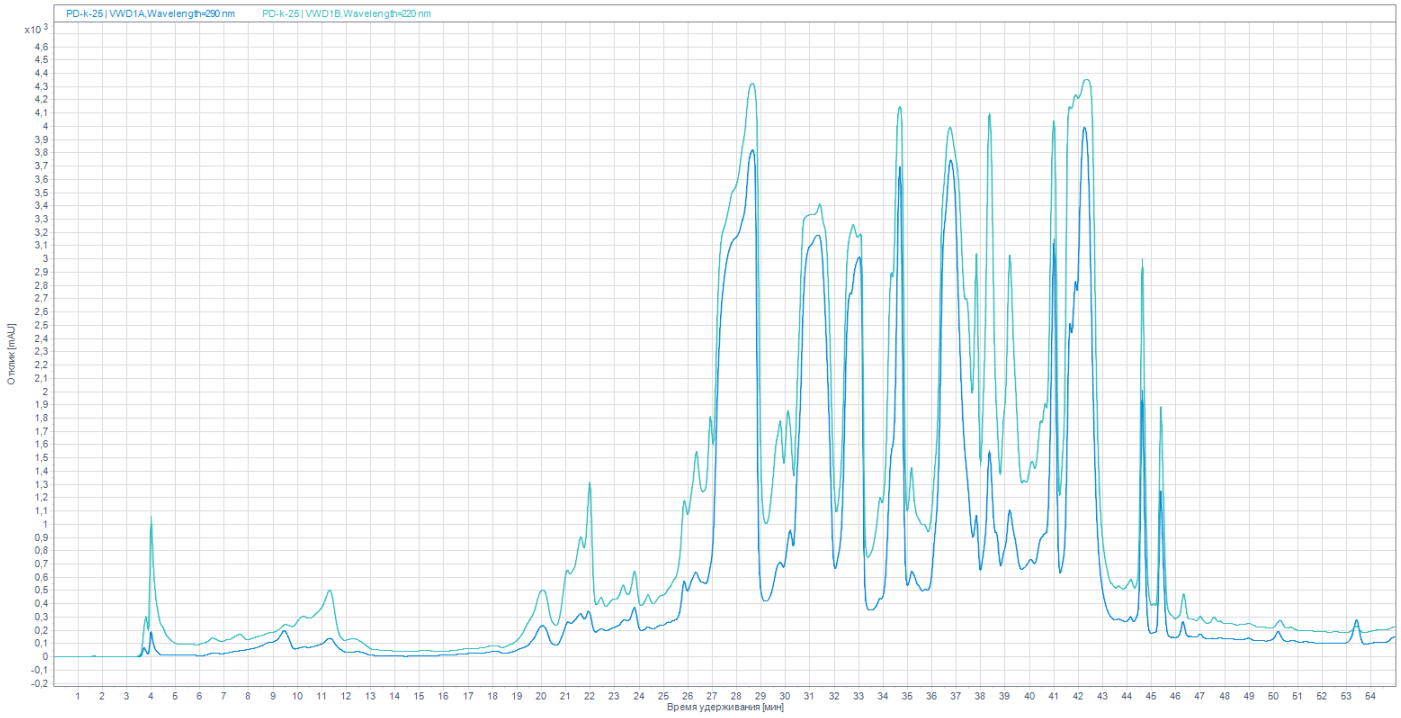

Figure S6. HPLC UV chromatogram of extract from fungus cultivated with 25 g/L sea salt concentration at 22 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

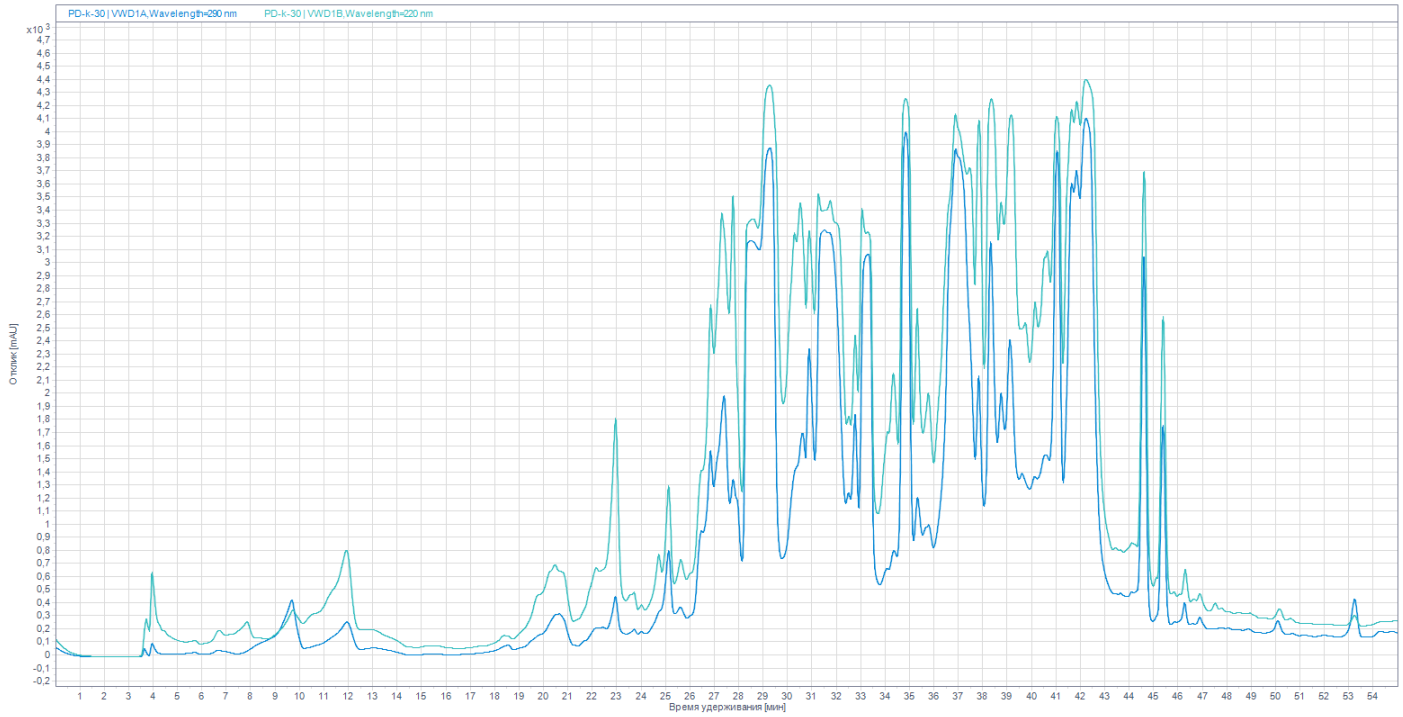

Figure S7. HPLC UV chromatogram of extract from fungus cultivated with 30 g/L sea salt concentration at 22 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

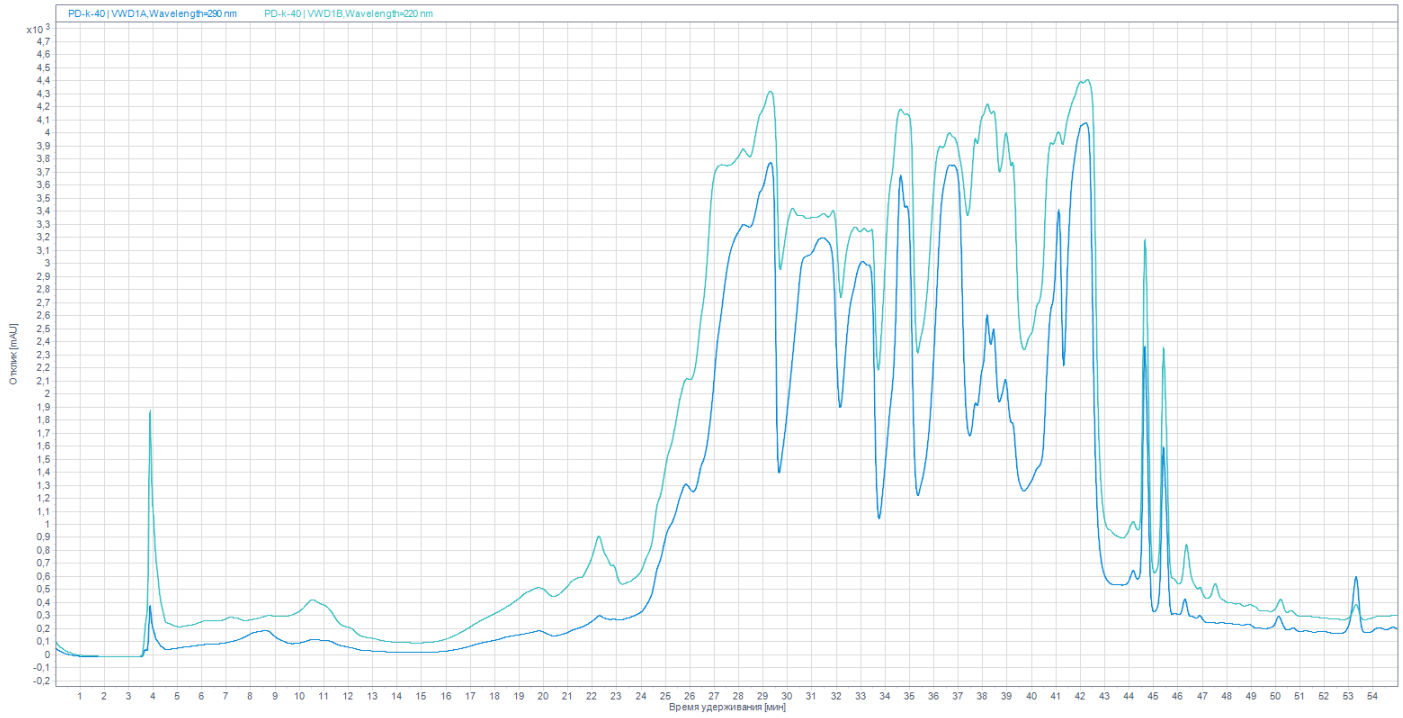

Figure S8. HPLC UV chromatogram of extract from fungus cultivated with 40 g/L sea salt concentration at 22 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

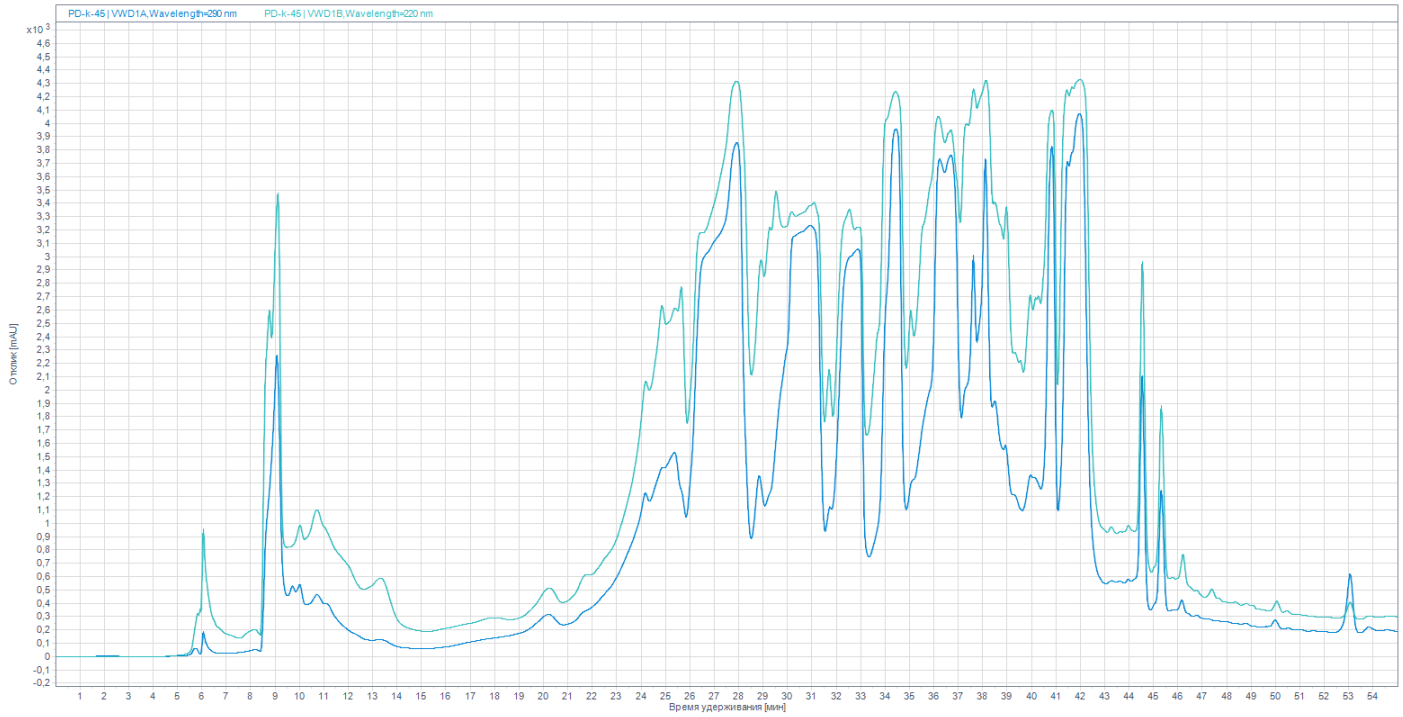

Figure S9. HPLC UV chromatogram of extract from fungus cultivated with 45 g/L sea salt concentration at 22 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

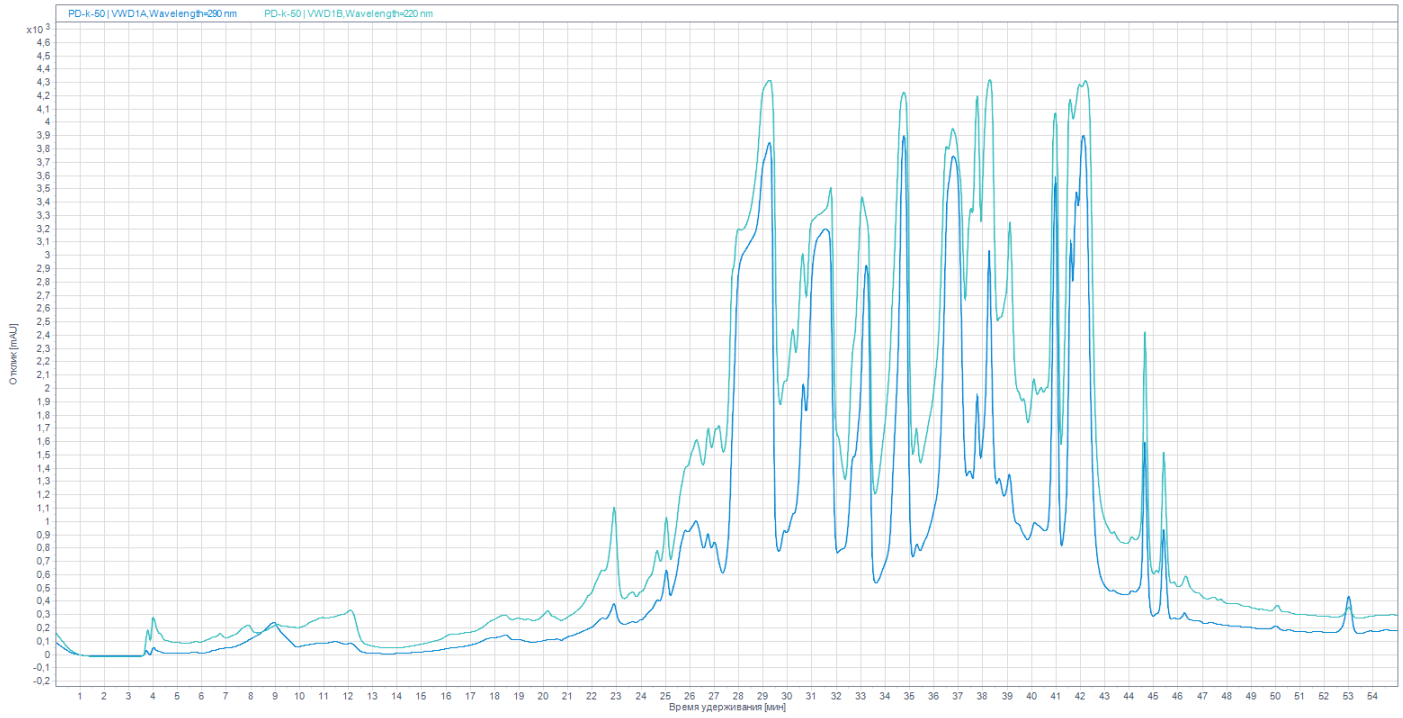

Figure S10. HPLC UV chromatogram of extract from fungus cultivated with 50 g/L sea salt concentration at 22 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

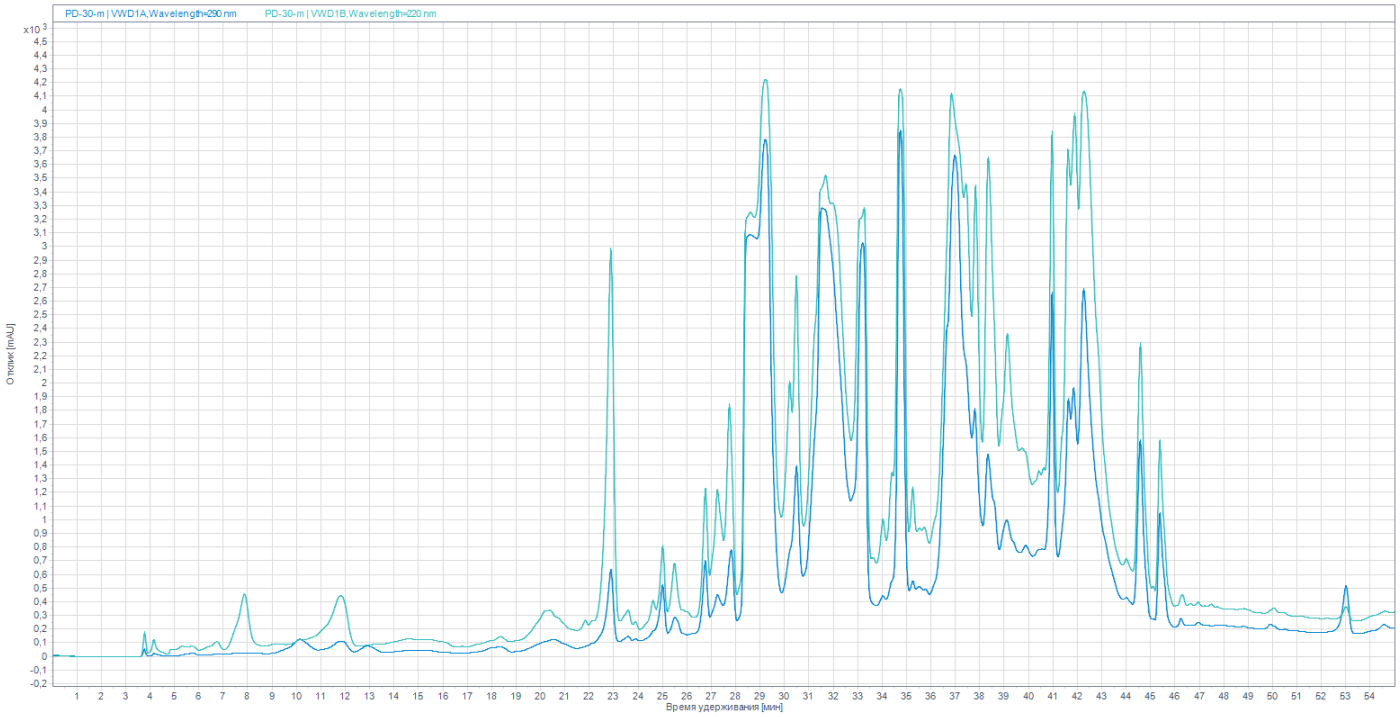

Figure S11. HPLC UV chromatogram of extract from fungus cultivated with sea water at 30 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

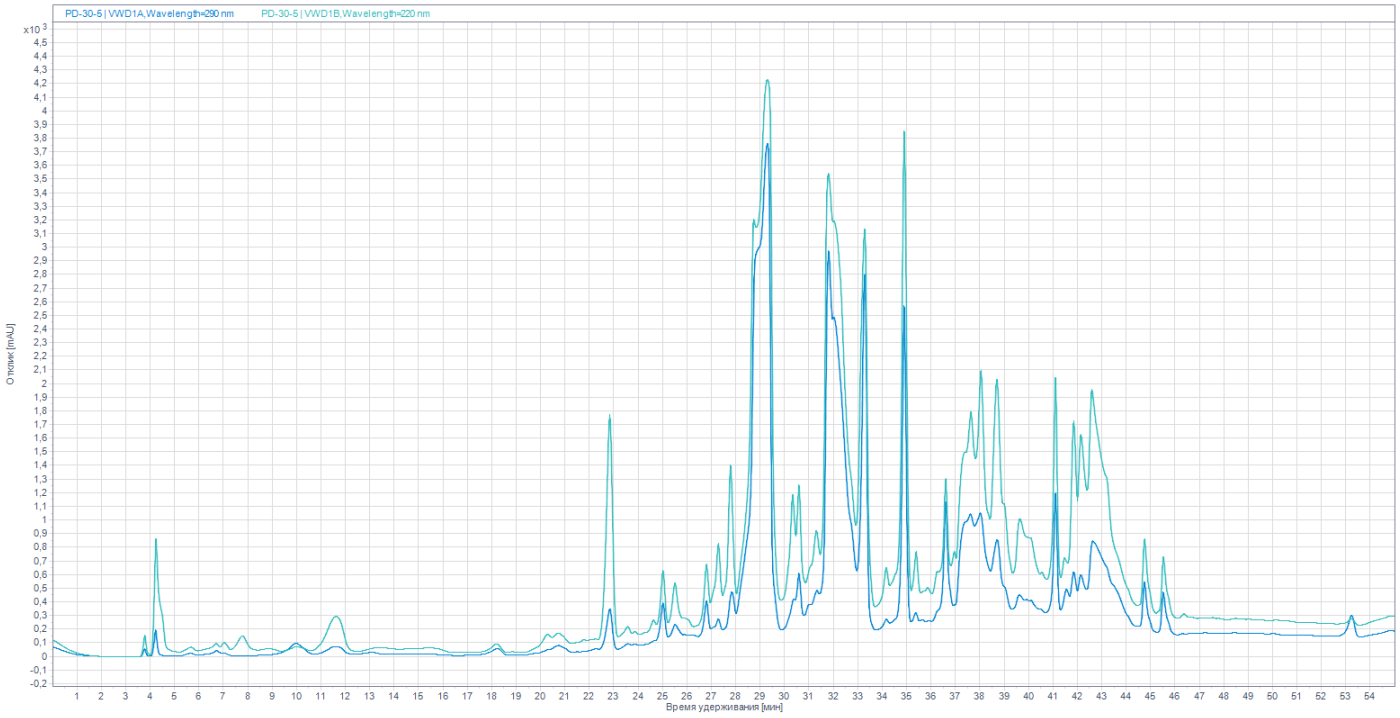

Figure S12. HPLC UV chromatogram of extract from fungus cultivated with 5 g/L sea salt concentration at 30 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

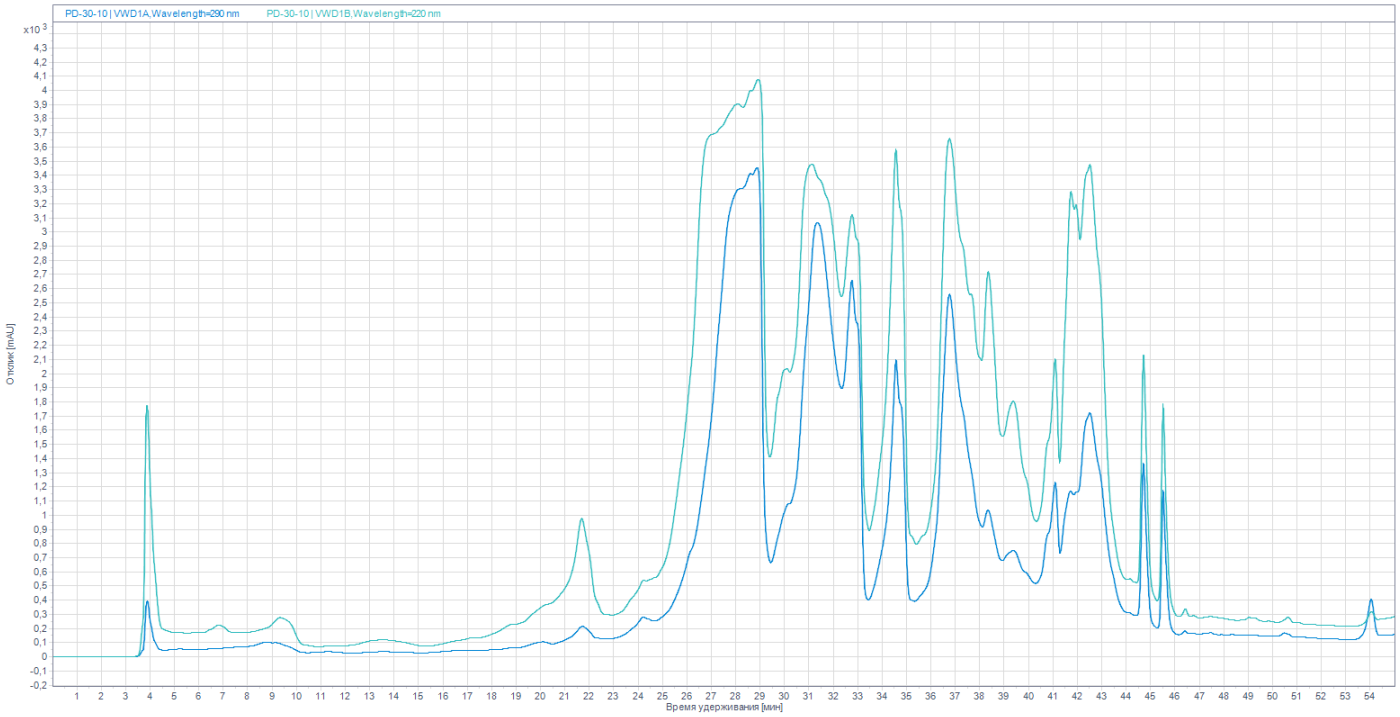

Figure S13. HPLC UV chromatogram of extract from fungus cultivated with 10 g/L sea salt concentration at 30 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

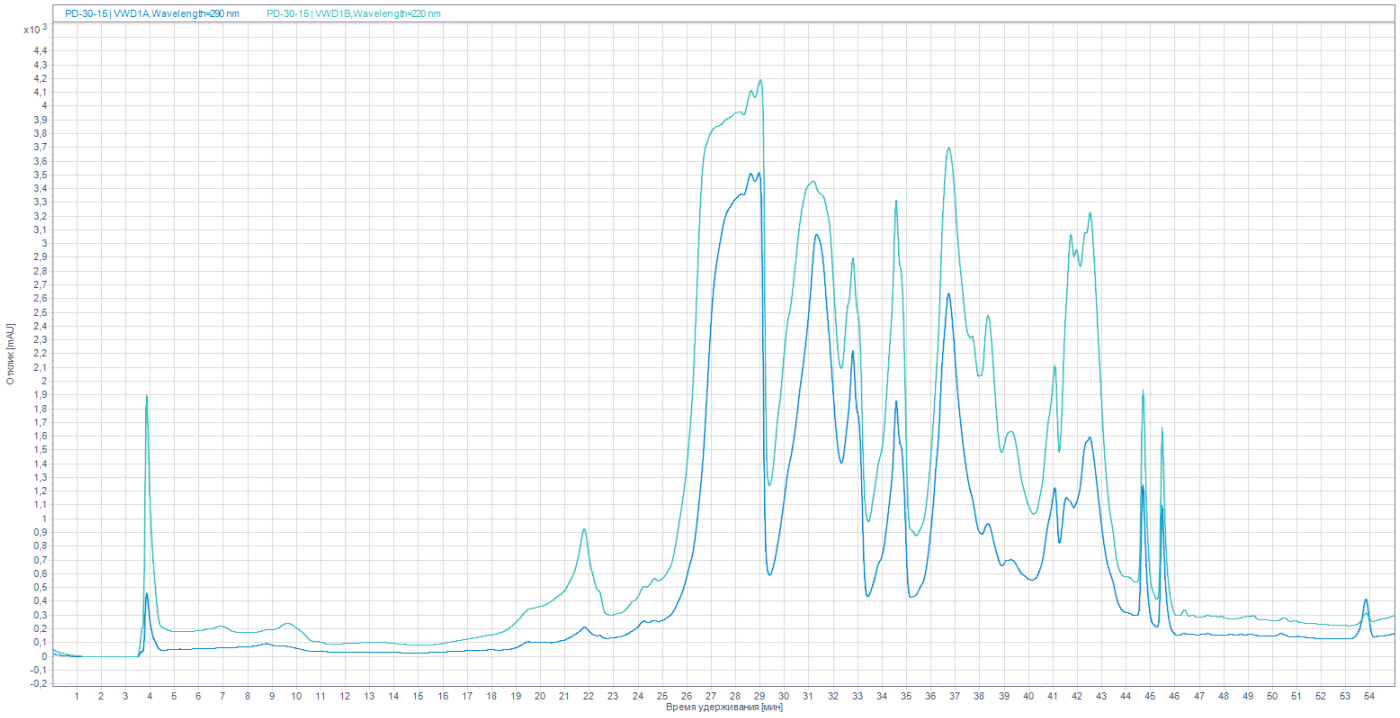

Figure S14. HPLC UV chromatogram of extract from fungus cultivated with 15 g/L sea salt concentration at 30 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

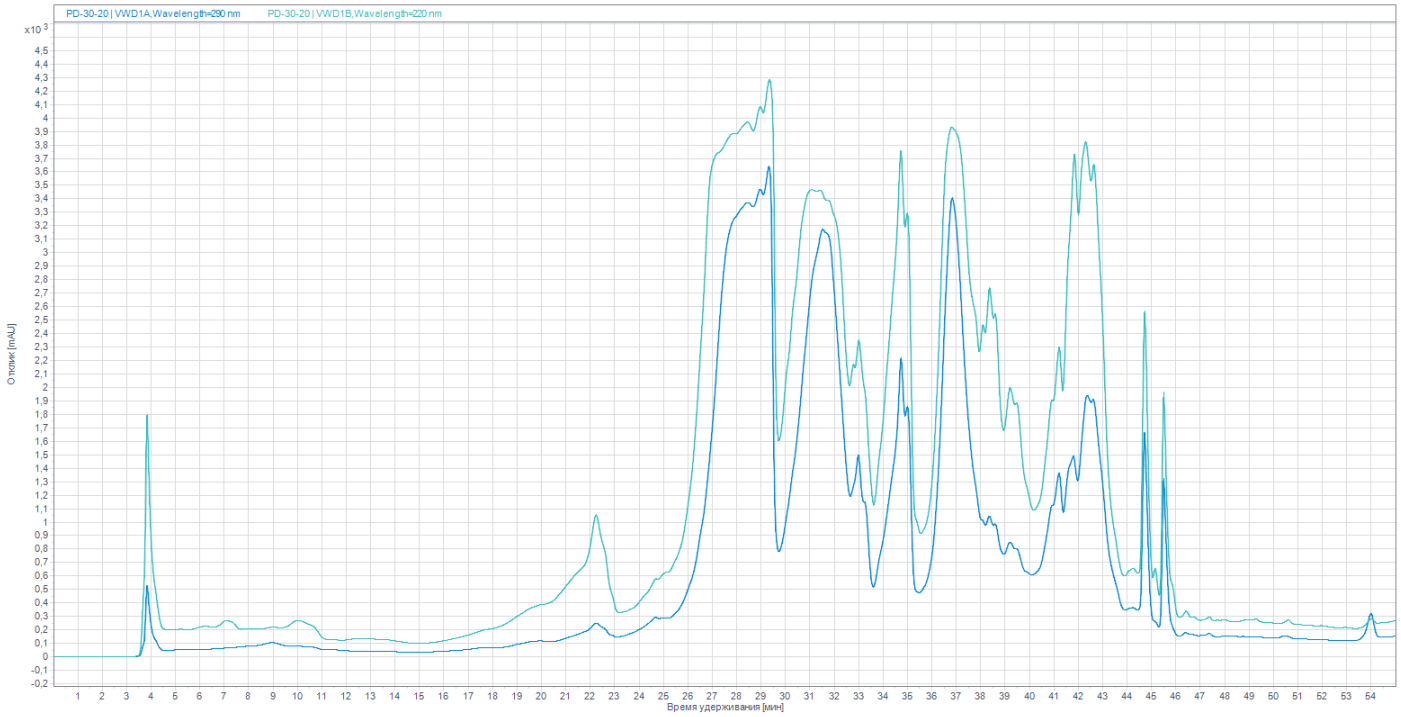

Figure S15. HPLC UV chromatogram of extract from fungus cultivated with 20 g/L sea salt concentration at 30 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

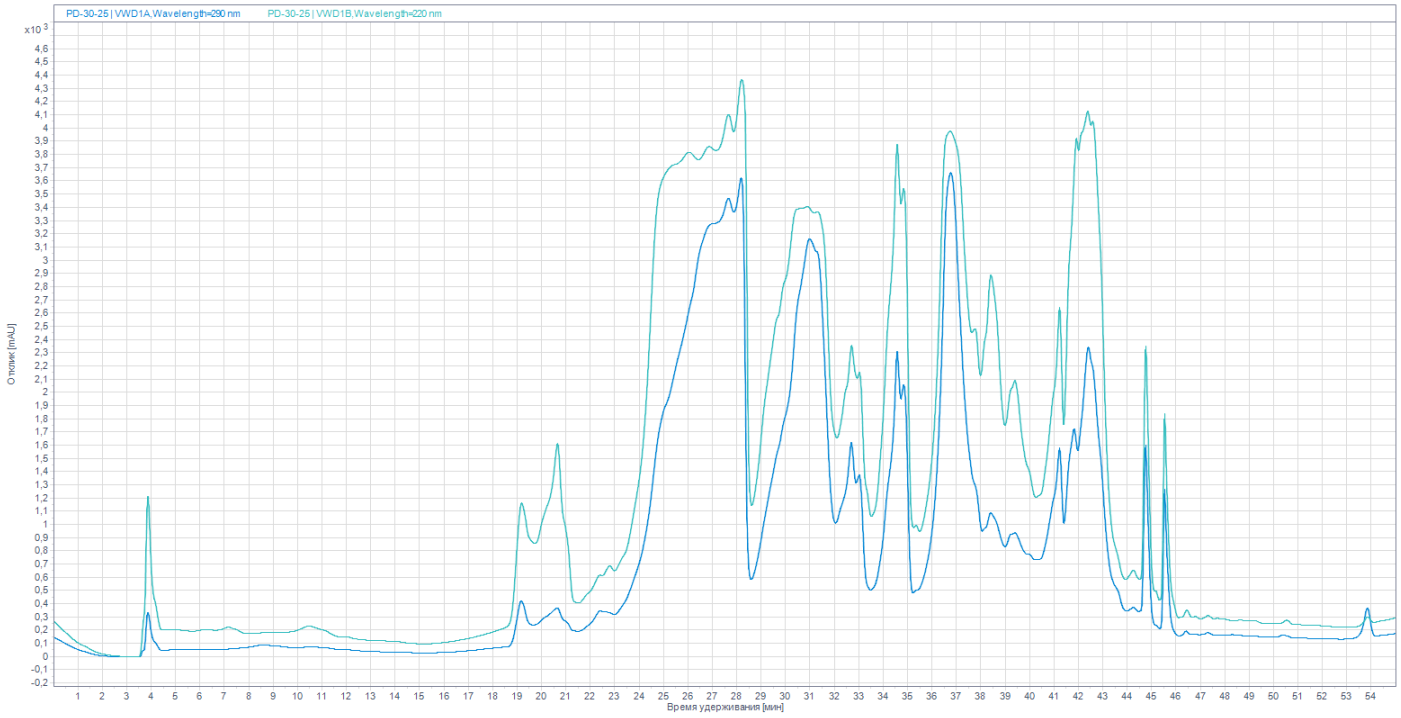

Figure S16. HPLC UV chromatogram of extract from fungus cultivated with 25 g/L sea salt concentration at 30 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

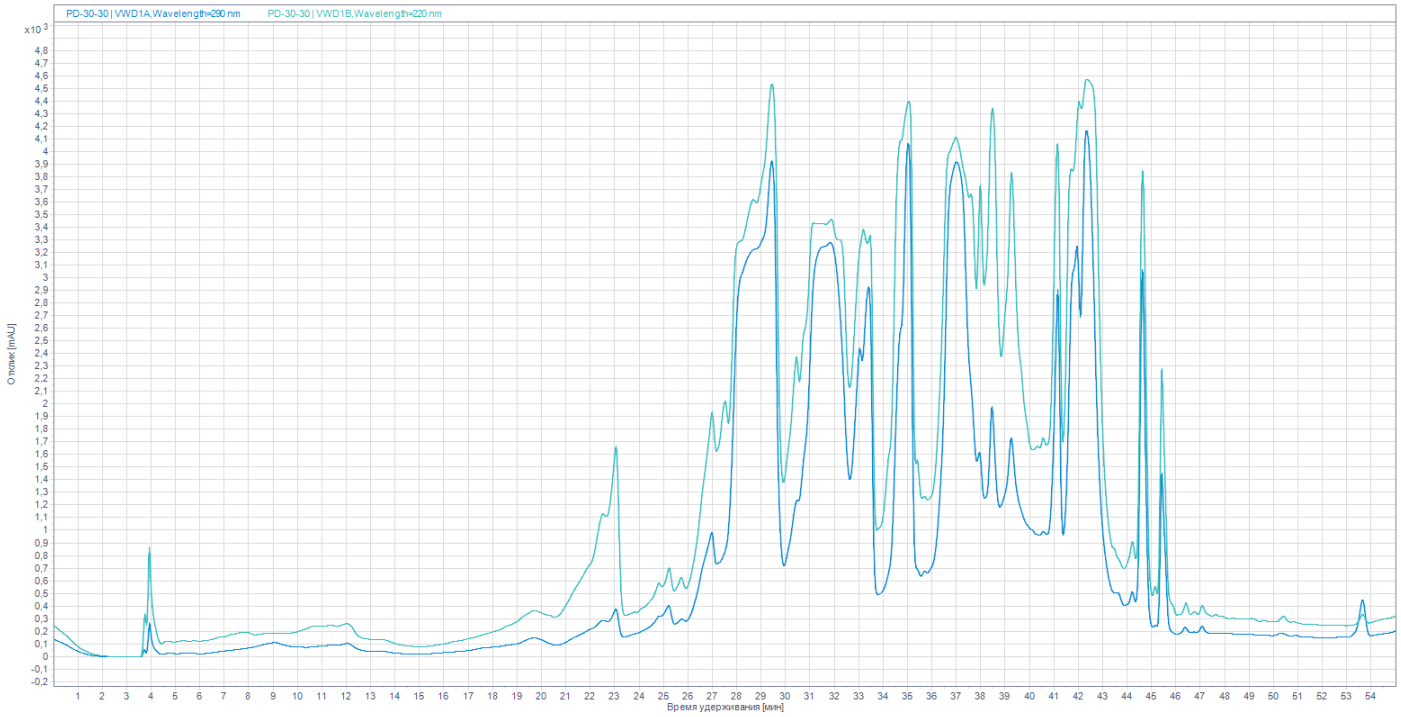

Figure S17. HPLC UV chromatogram of extract from fungus cultivated with 30 g/L sea salt concentration at 30 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

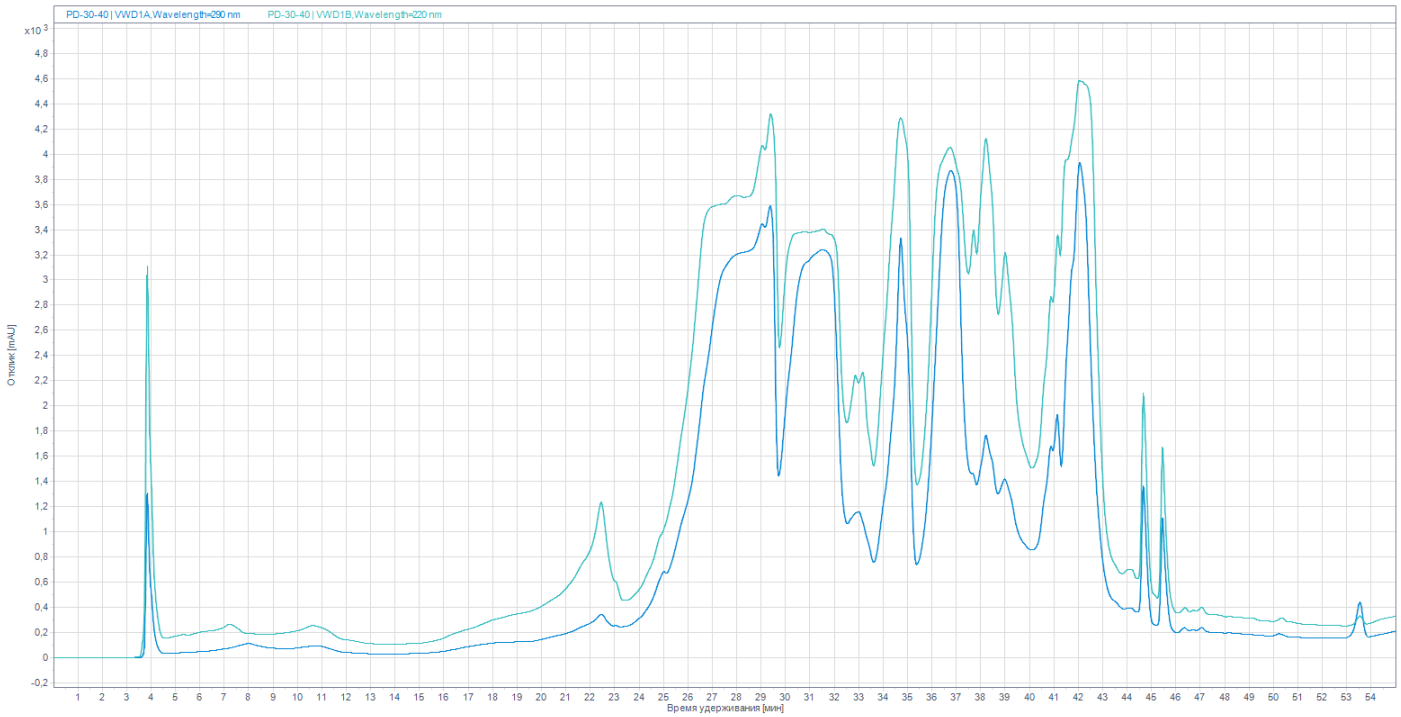

Figure S18. HPLC UV chromatogram of extract from fungus cultivated with 40 g/L sea salt concentration at 30 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

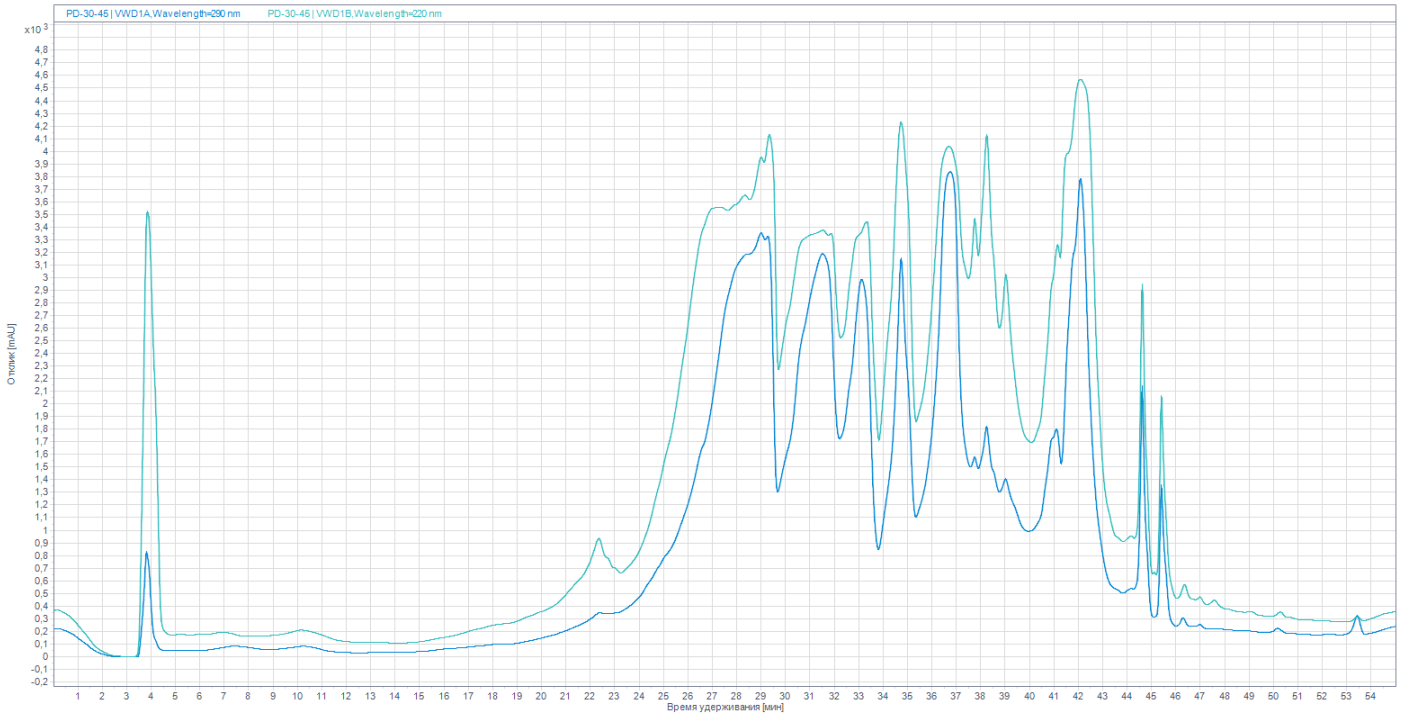

Figure S19. HPLC UV chromatogram of extract from fungus cultivated with 45 g/L sea salt concentration at 30 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

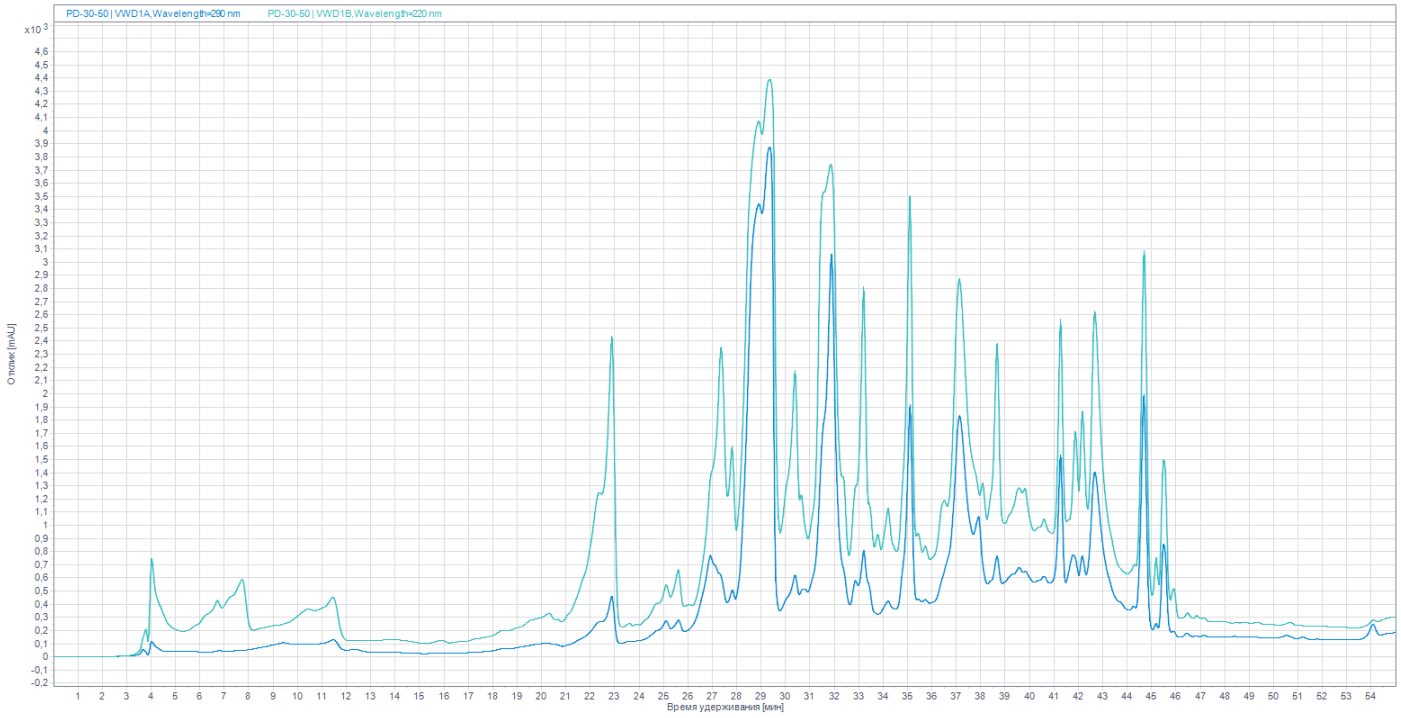

Figure S20. HPLC UV chromatogram of extract from fungus cultivated with 50 g/L sea salt concentration at 30 °C. Chromatograms were recorded at 290 nm (blue) and 220 nm (green).

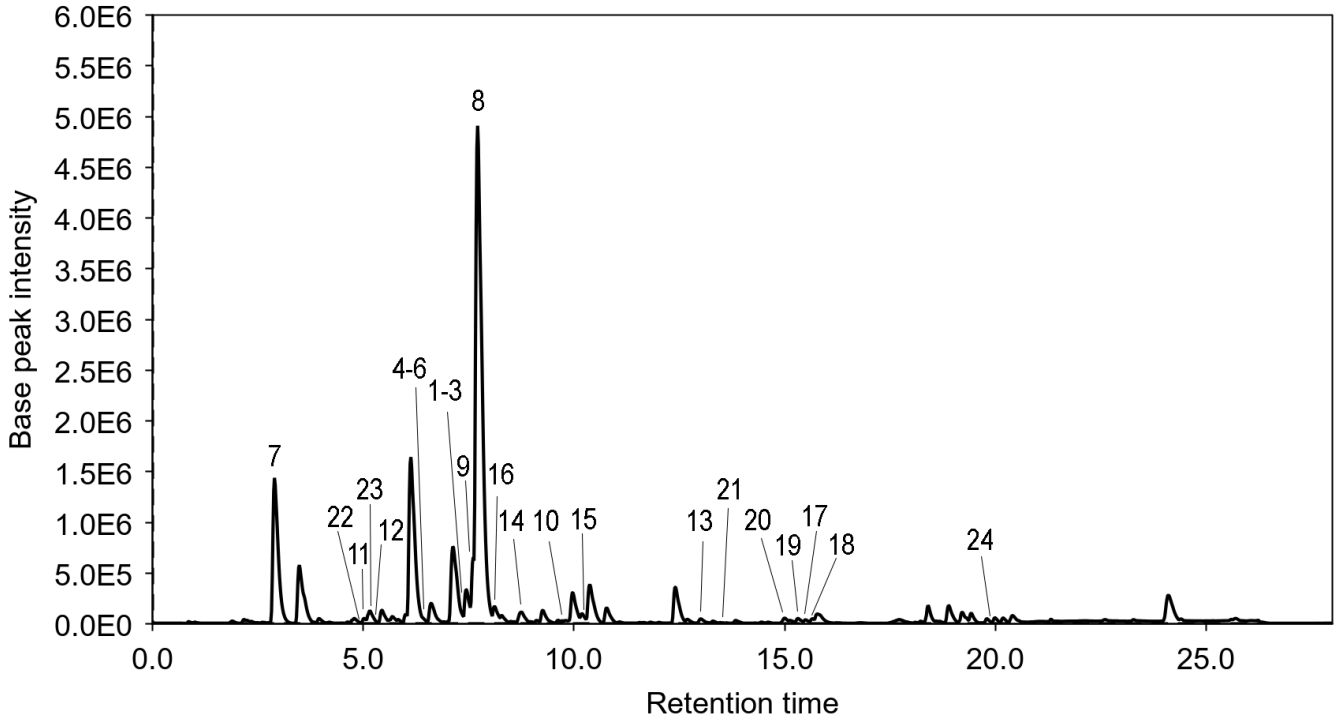

Figure S21. UHPLC MS chromatogram of extract from fungus cultivated with sea water at 22 °C.

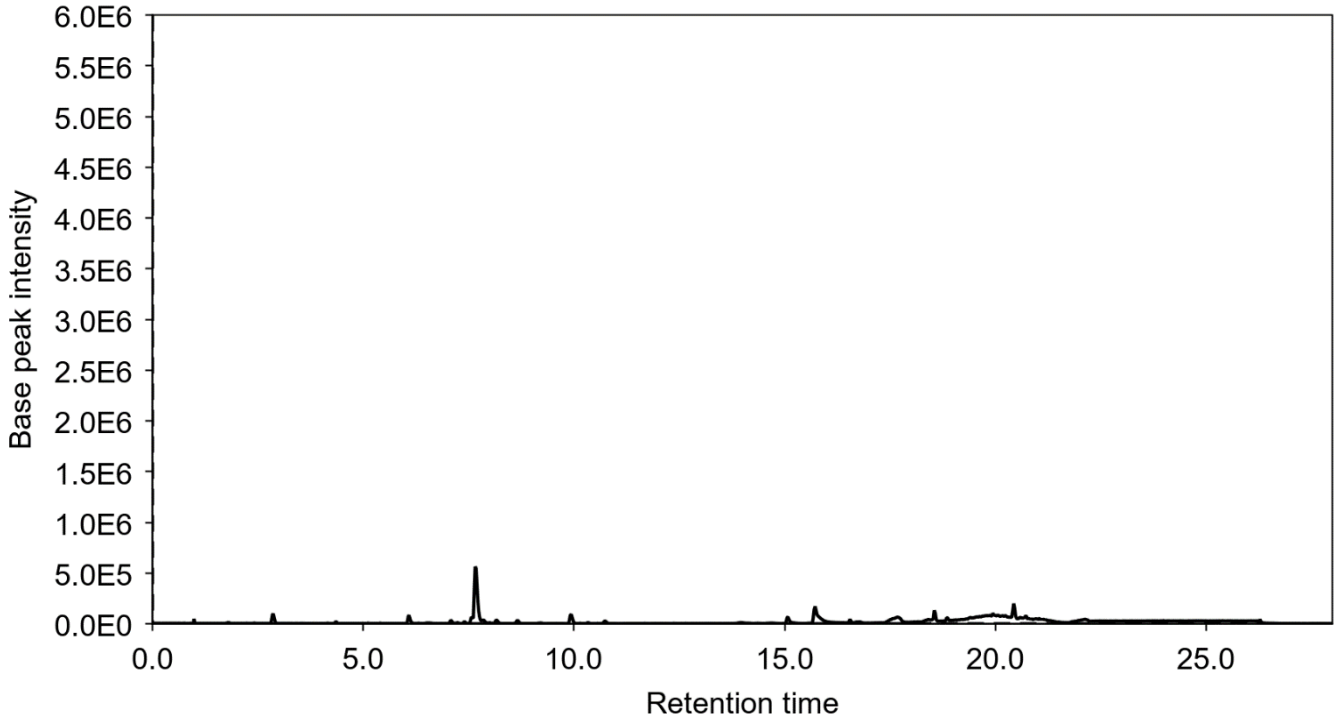

Figure S22. UHPLC MS chromatogram of extract from fungus cultivated with 5 g/L sea salt concentration at 22 °C.

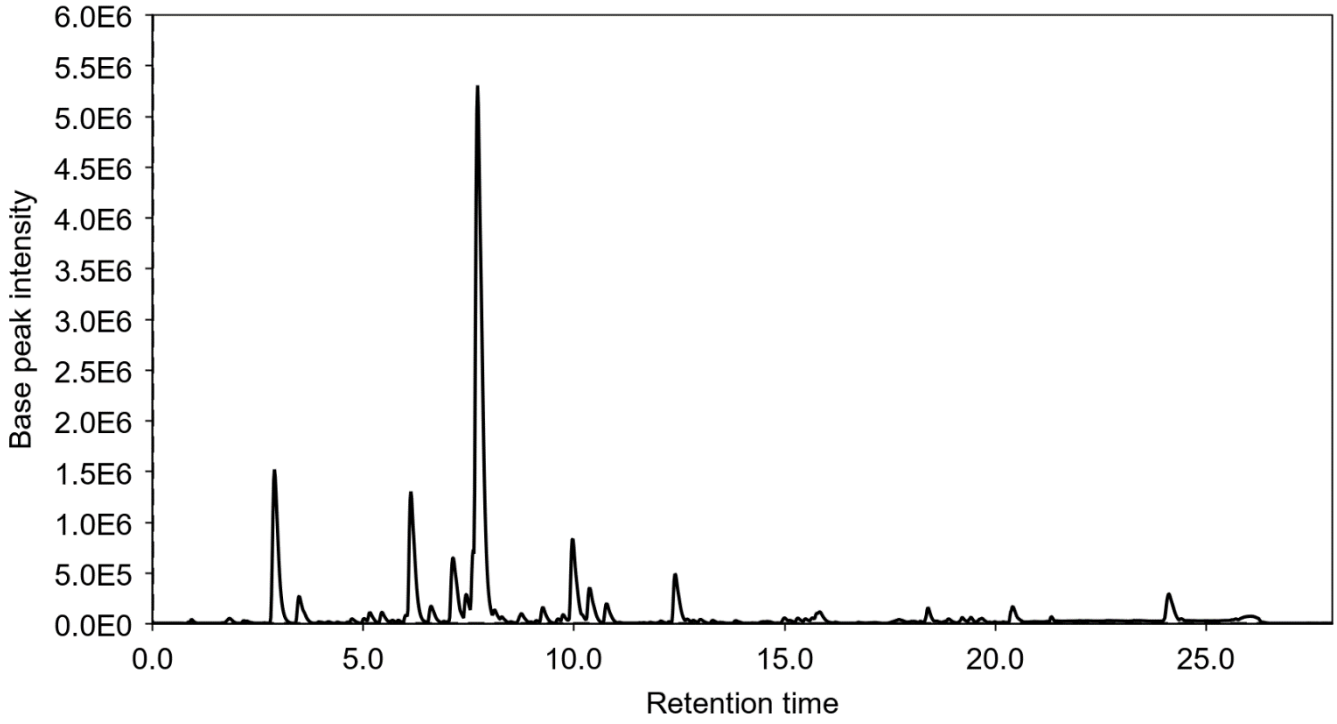

Figure S23. UHPLC MS chromatogram of extract from fungus cultivated with 15 g/L sea salt concentration at 22 °C.

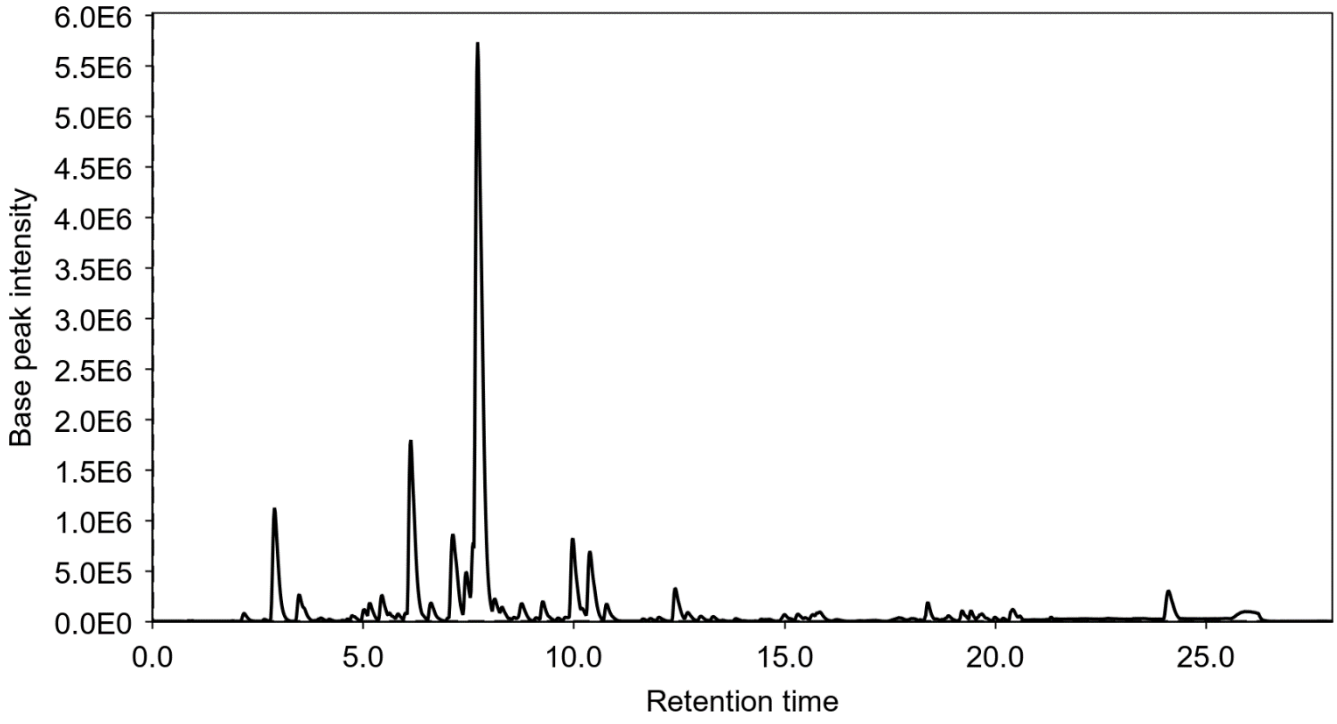

Figure S24. UHPLC MS chromatogram of extract from fungus cultivated with 50 g/L sea salt concentration at 22 °C.

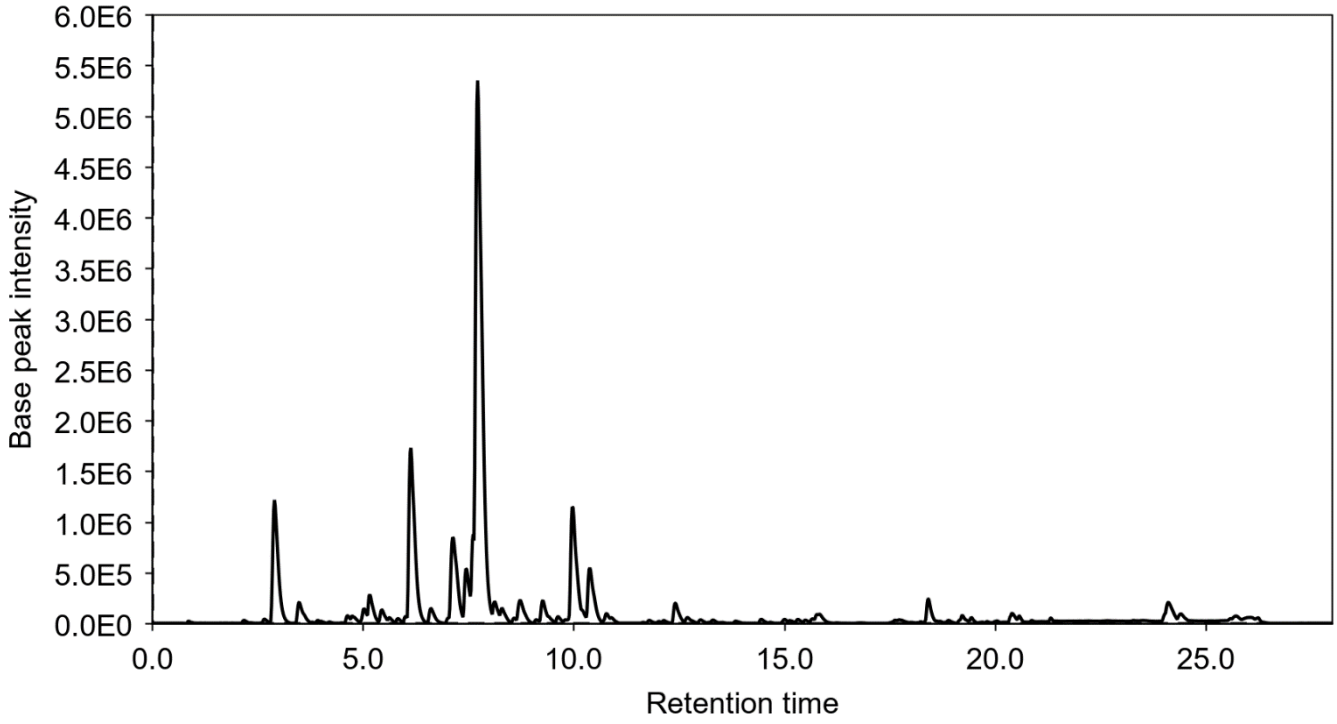

Figure S25. UHPLC MS chromatogram of extract from fungus cultivated with sea water at 30 °C.

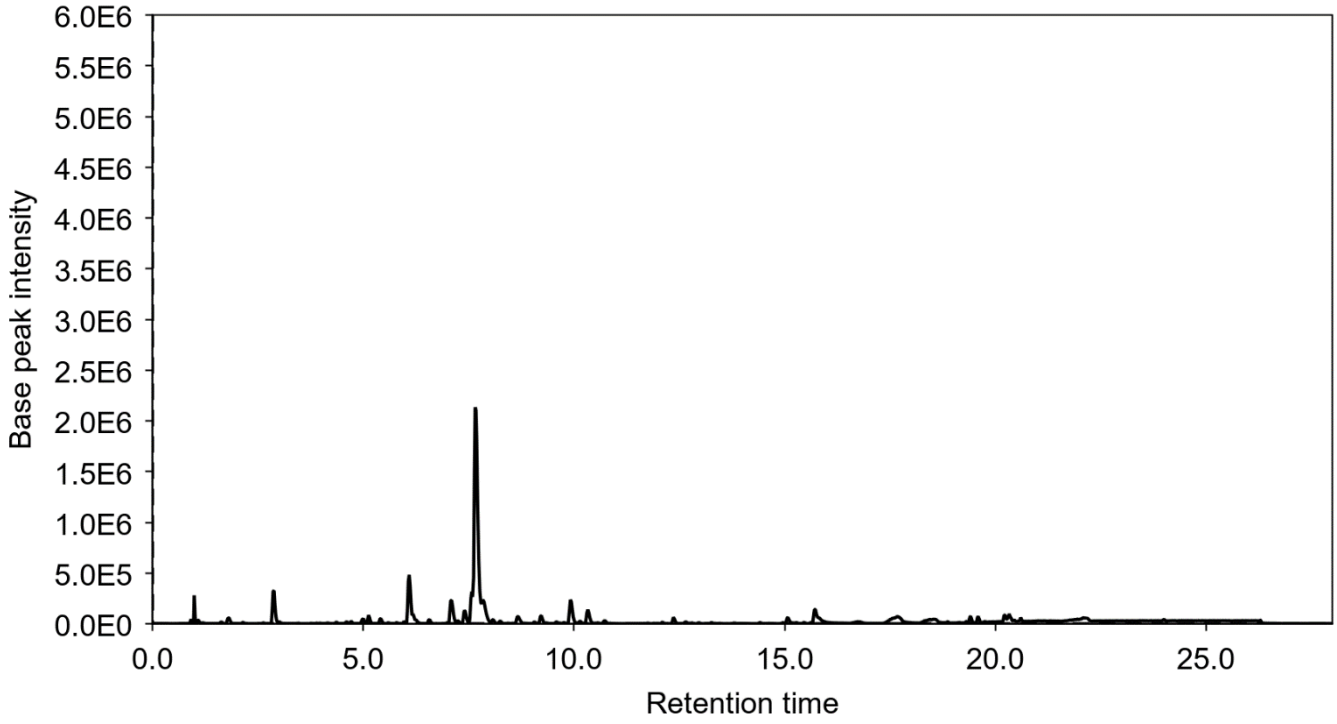

Figure S26. UHPLC MS chromatogram of extract from fungus cultivated with 5 g/L sea salt concentration at 30 °C.

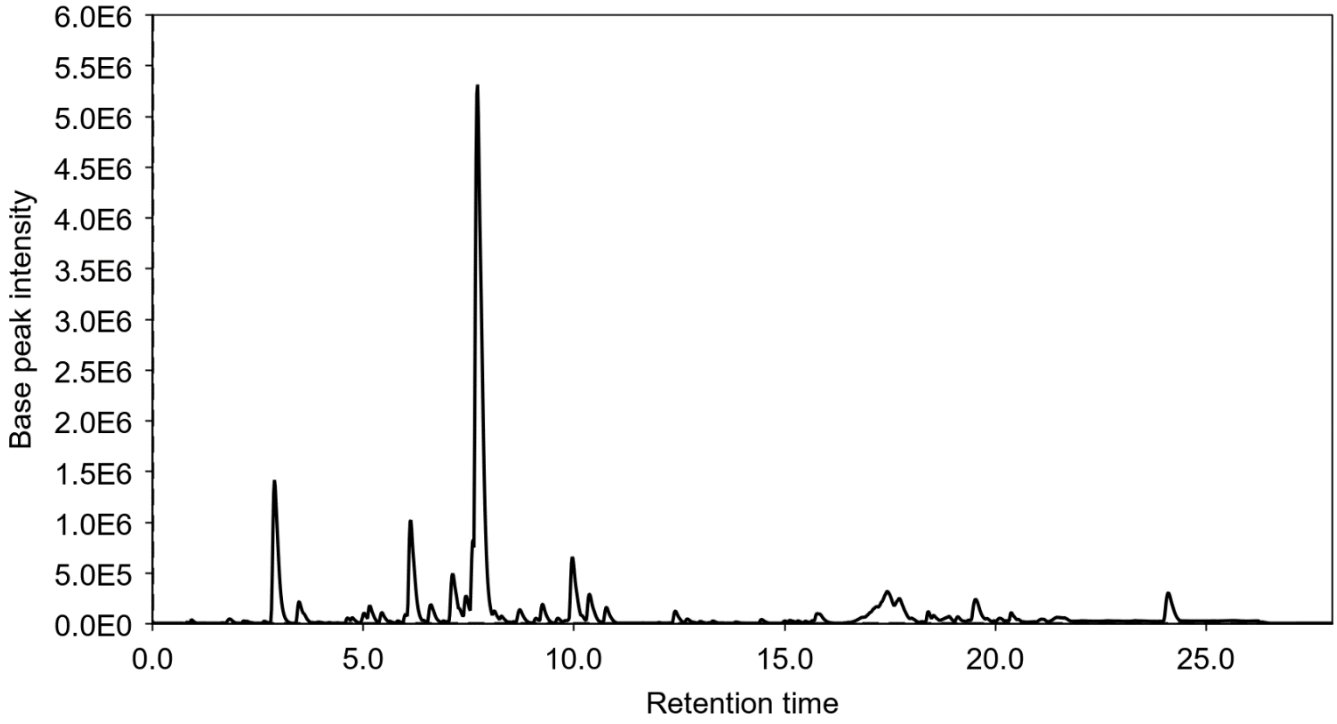

Figure S27. UHPLC MS chromatogram of extract from fungus cultivated with 15 g/L sea salt concentration at 30 °C.

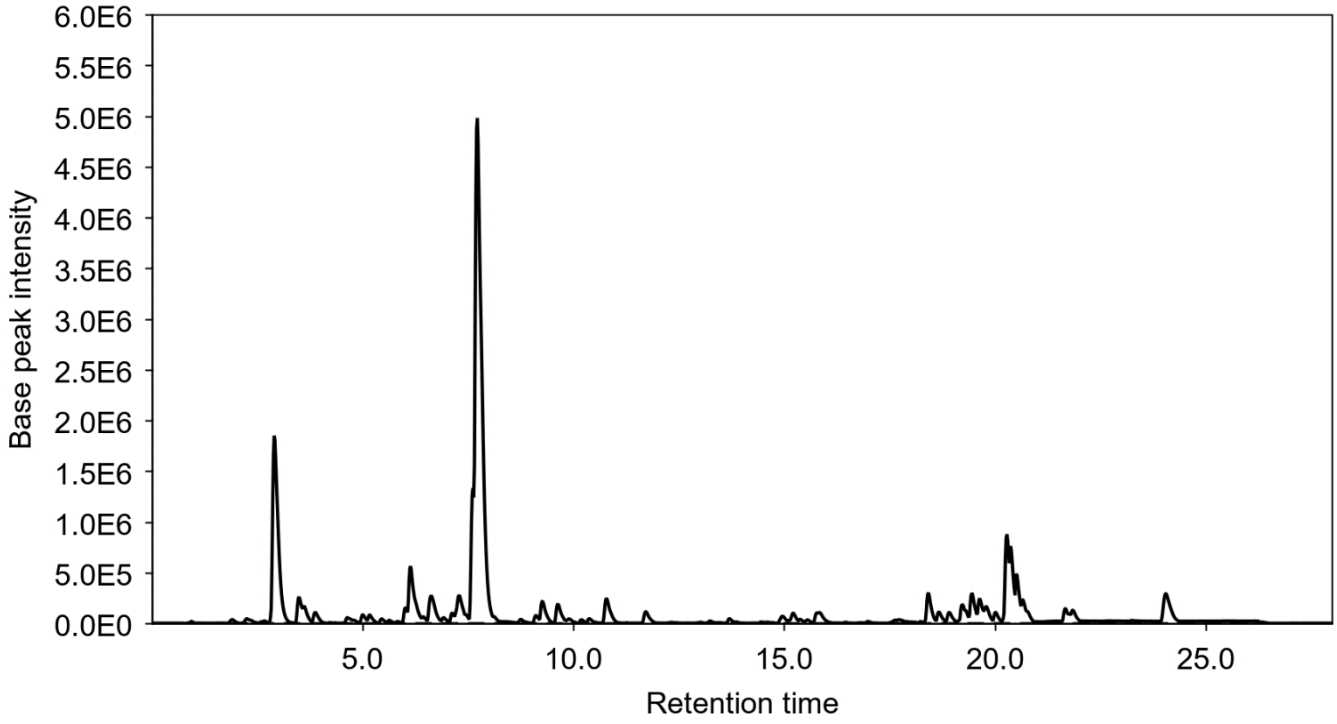

Figure S28. UHPLC MS chromatogram of extract from fungus cultivated with 50 g/L sea salt concentration at 30 °C.

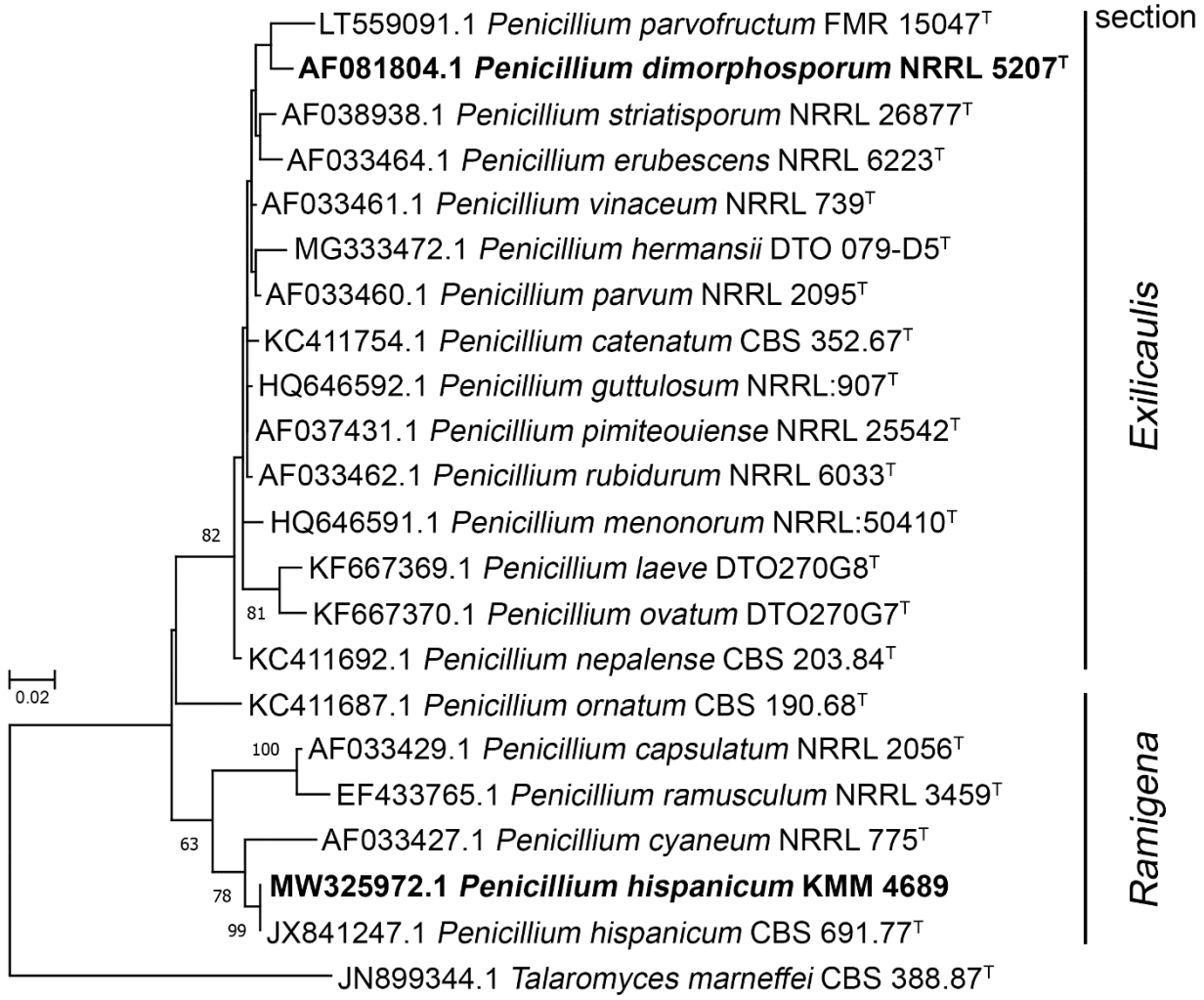

Figure S29. ML tree based on ITS region sequences showing phylogenetic position of the strain KMM 4689 among members of genus *Penicillium* section *Ramigena* and section *Exilicaulis*. Bootstrap values (%) of 1000 replications. Nodes with confidence values greater than 50% are indicated. The scale bars represent 0.02 substitutions per site.

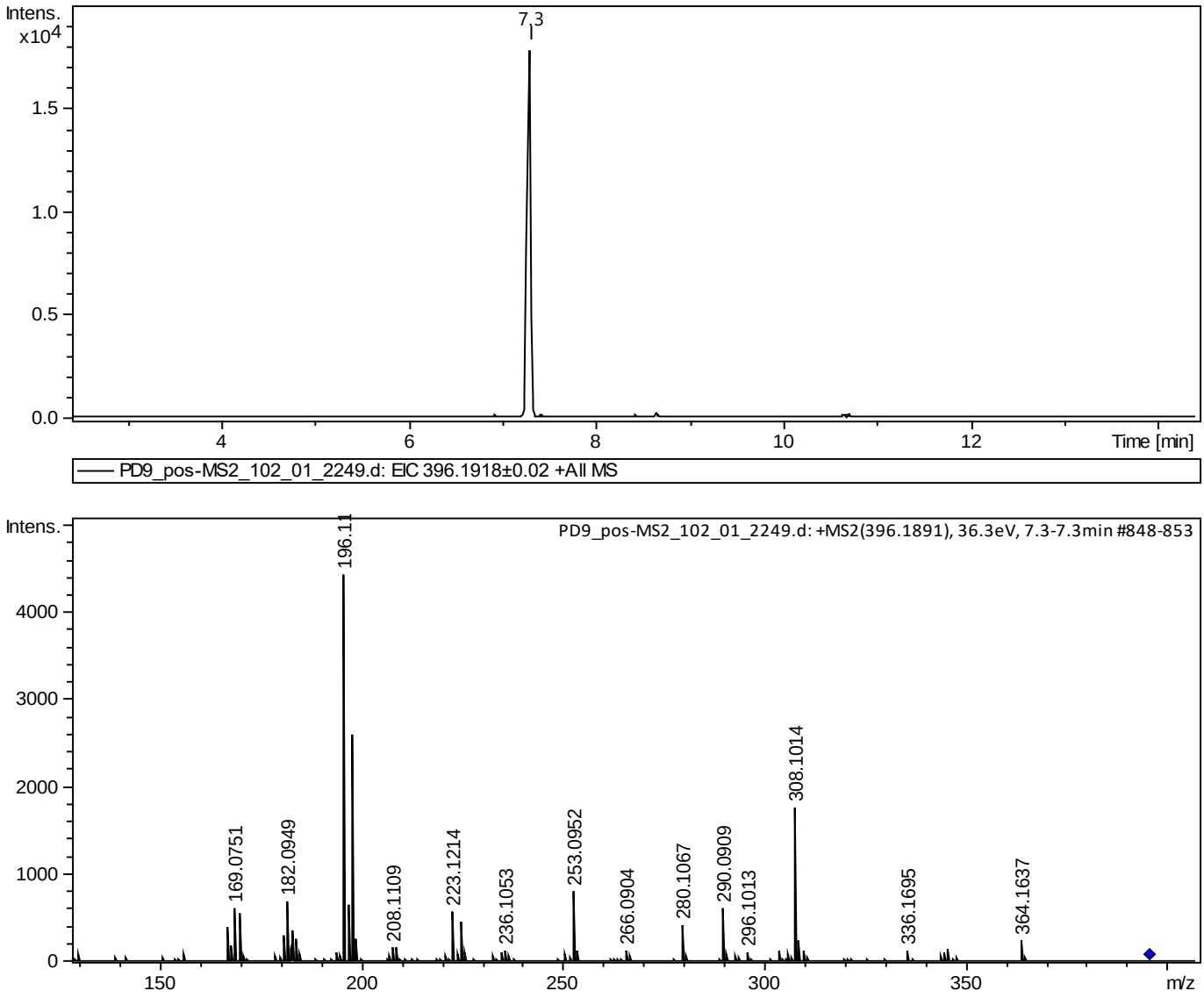

Figure S30. HPLC MS retention time and MS/MS of 16*α*-hydroxy-17β-methoxy-deoxydihydroisoaustamide (**1**) (reference compound)

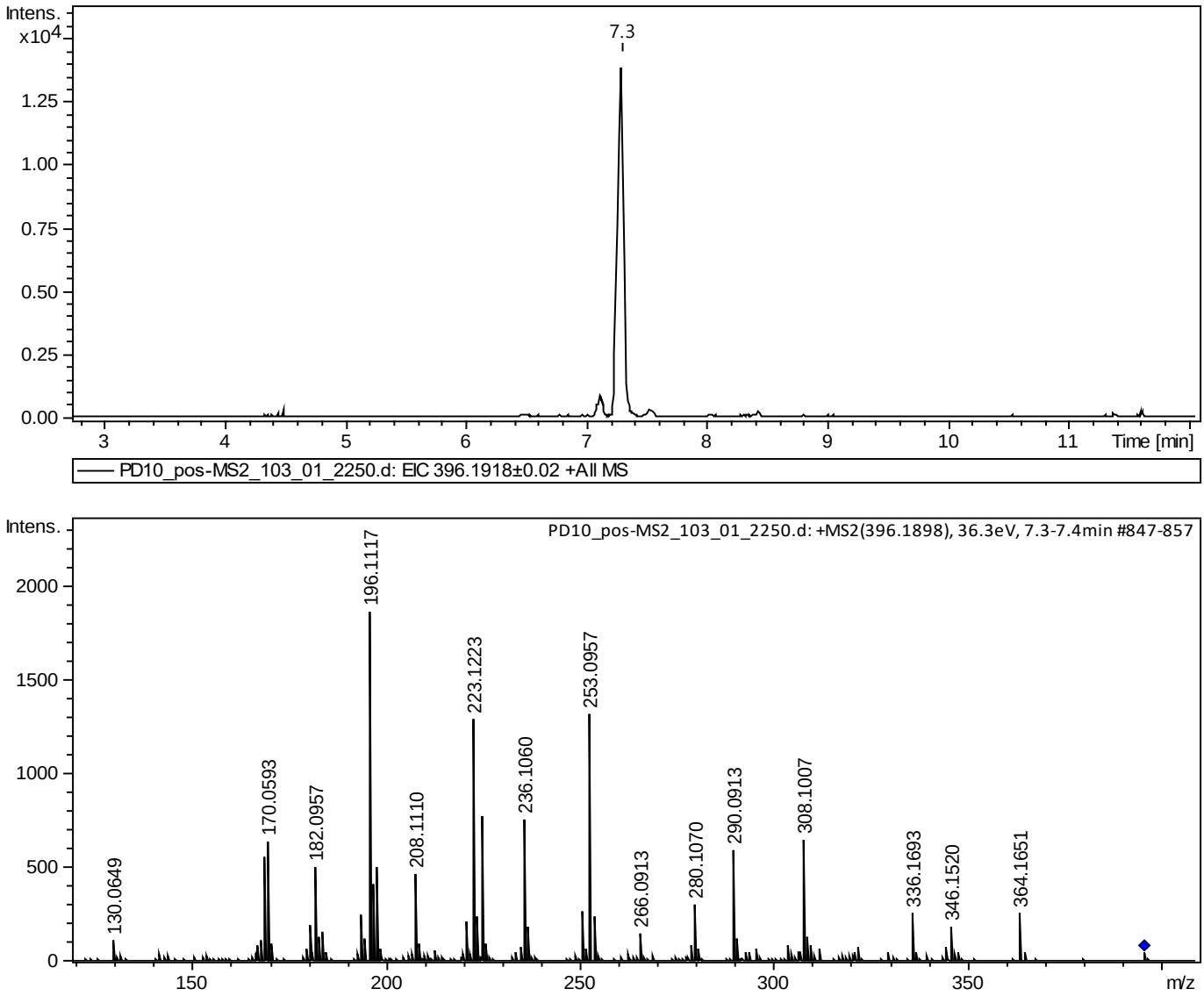

Figure S31. HPLC MS retention time and MS/MS of 16β-hydroxy-17α-methoxy-deoxydihydroisoaustamide (**2**) (reference compound)

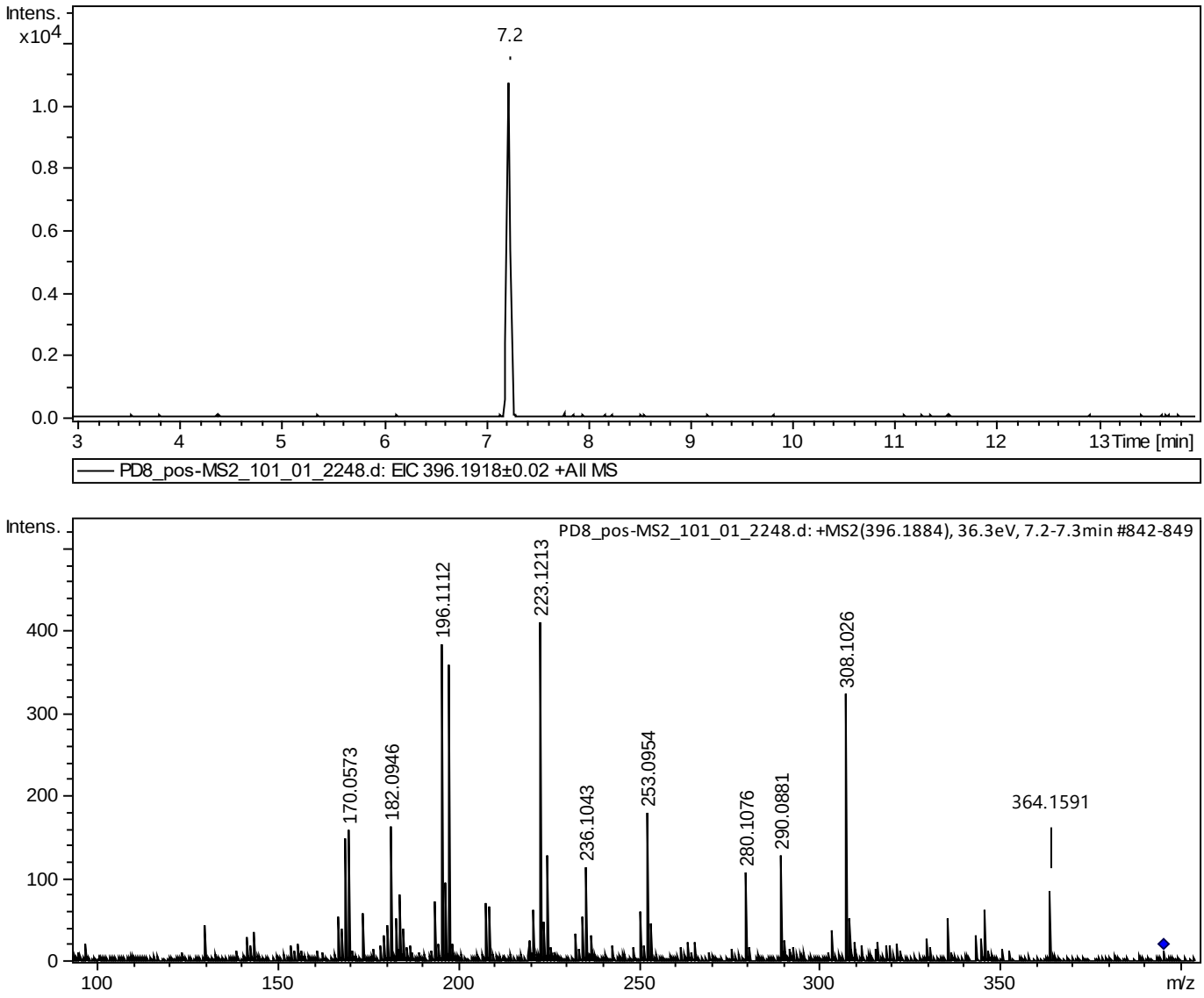

Figure S32. HPLC MS retention time and MS/MS of 16α-hydroxy-17α-methoxy-deoxydihydroisoaustamide (**3**) (reference compound)

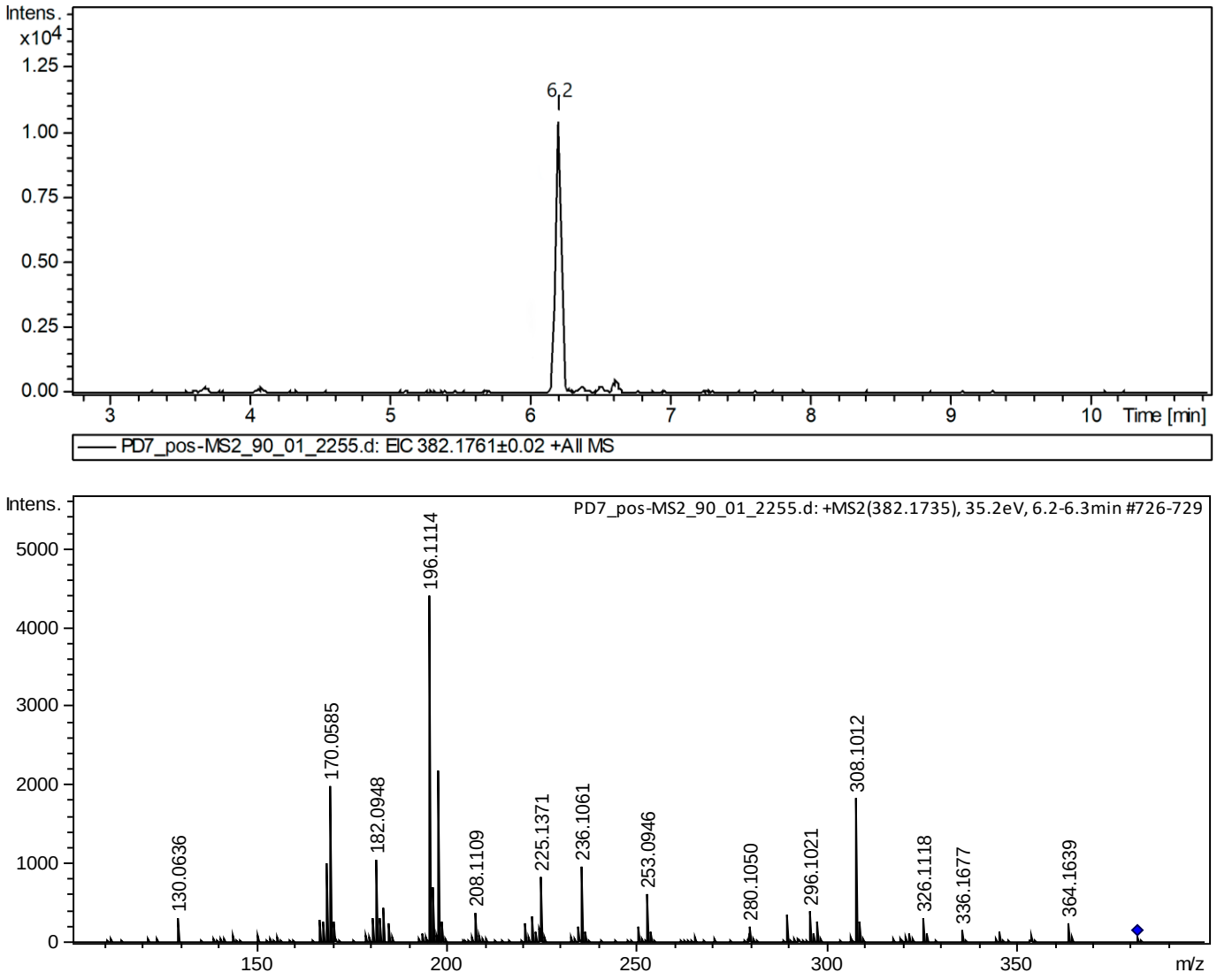

Figure S33. HPLC MS retention time and MS/MS of 16,17-dihydroxy-deoxydihydroisoaustamide (**4**) (reference compound)

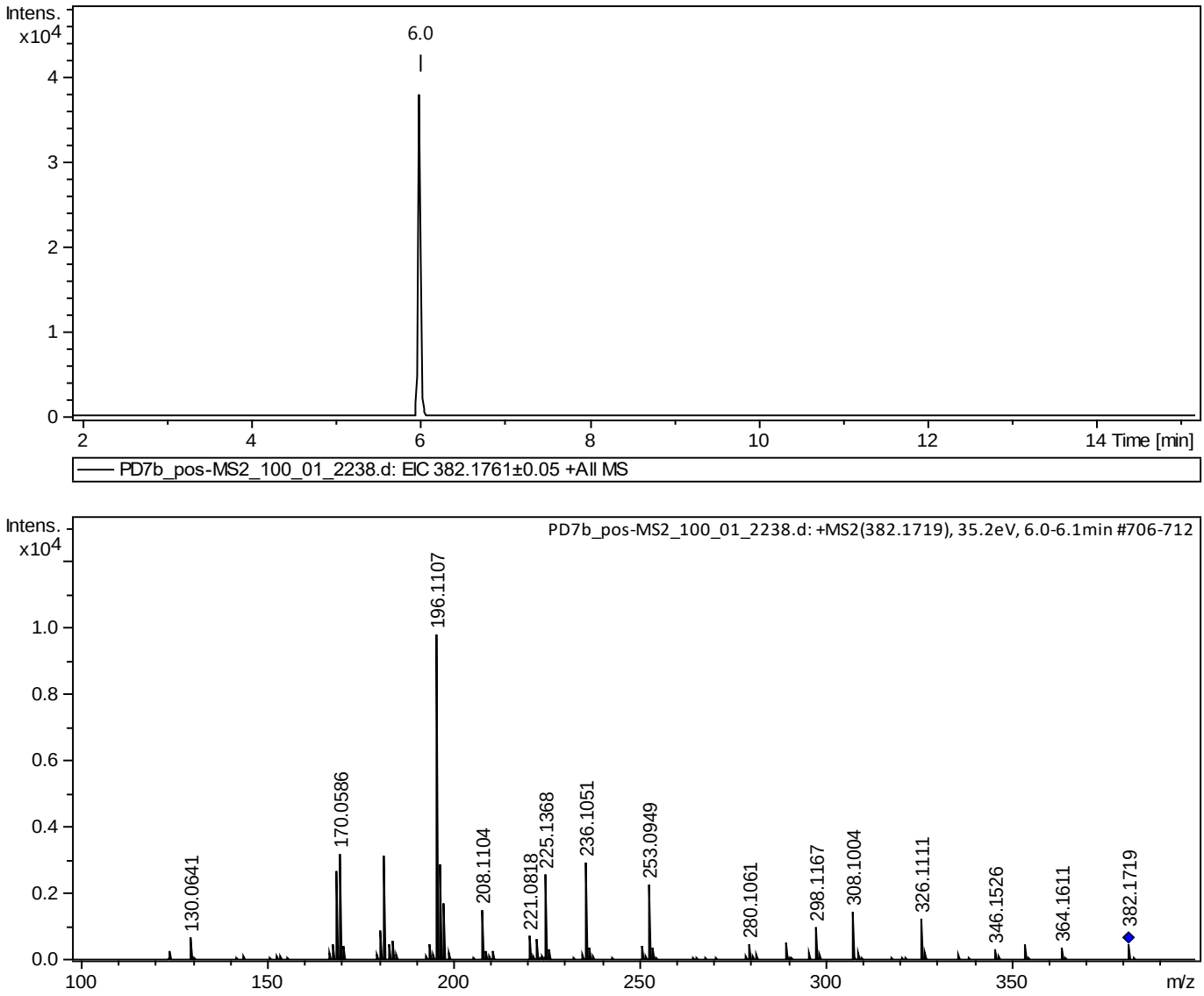

Figure S34. HPLC MS retention time and MS/MS of 16β,17α-dihydroxy-deoxydihydroisoaustamide (**5**) (reference compound)

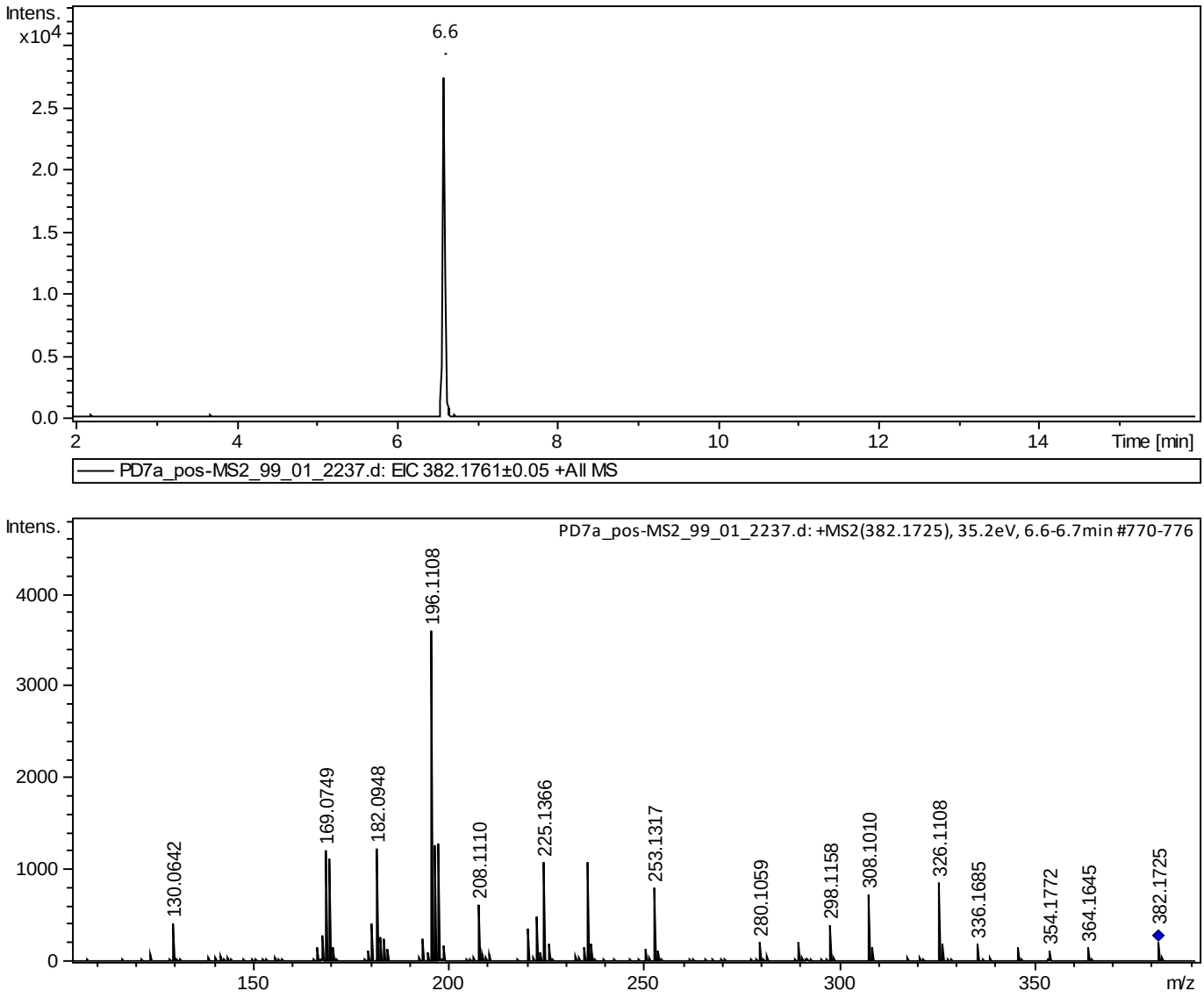

Figure S35. HPLC MS retention time and MS/MS of 16α,17α-dihydroxy-deoxydihydroisoaustamide (**6**) (reference compound)

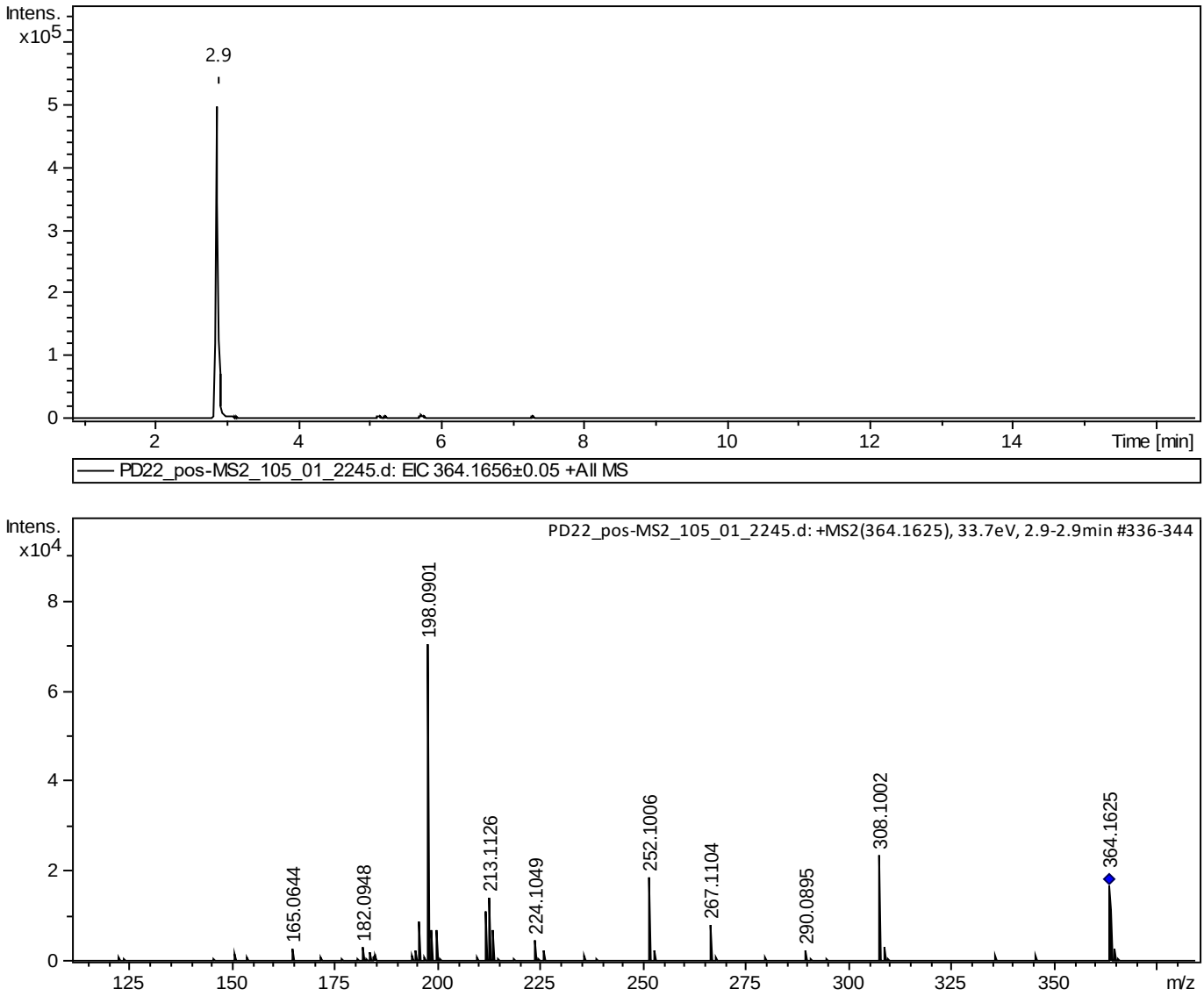

Figure S36. HPLC MS retention time and MS/MS of 3β-hydroxy-deoxyisoaustamide (**7**) (reference compound)

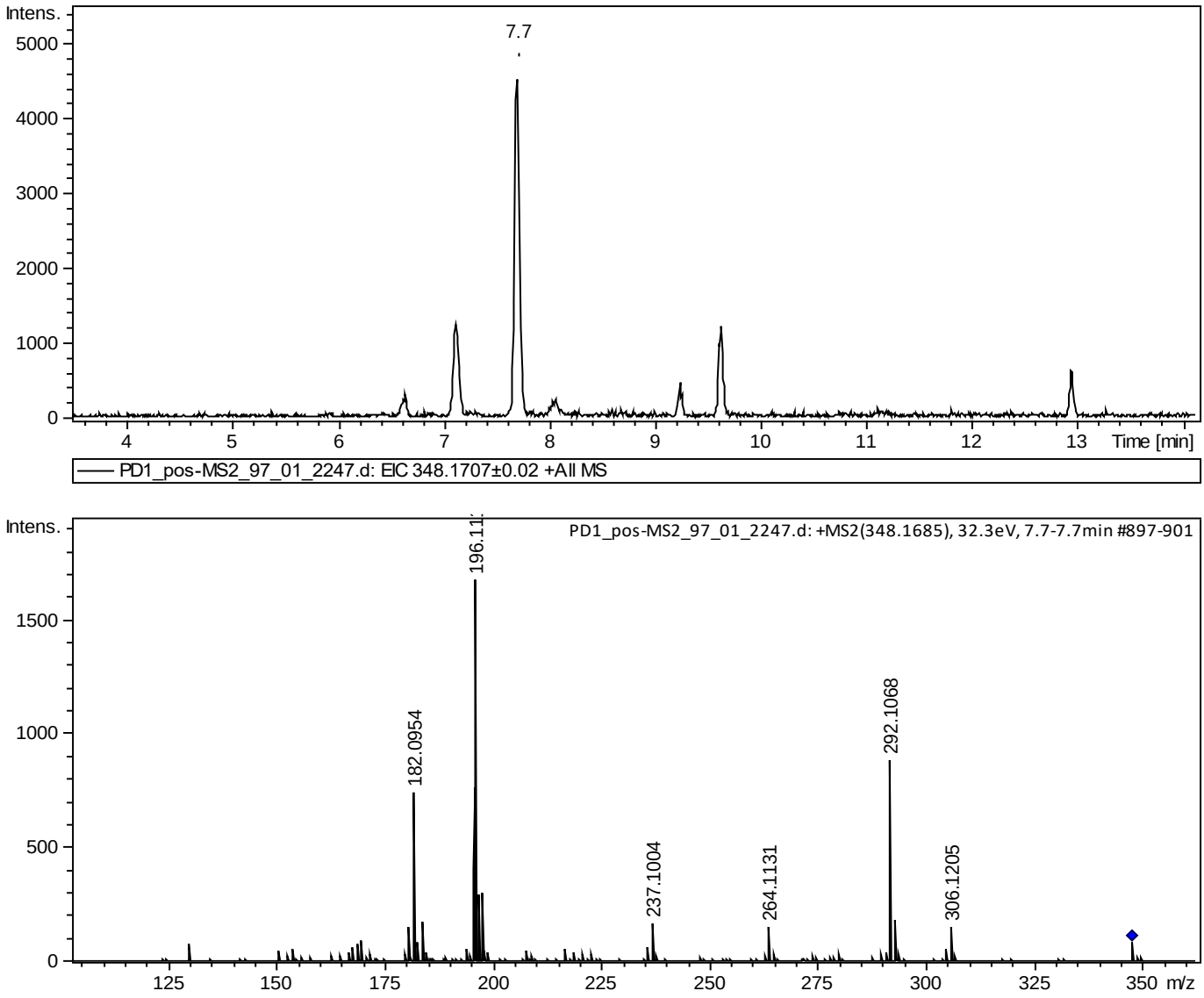

Figure S37. HPLC MS retention time and MS/MS of (+)-deoxyisoaustamide (**8**) (reference compound)

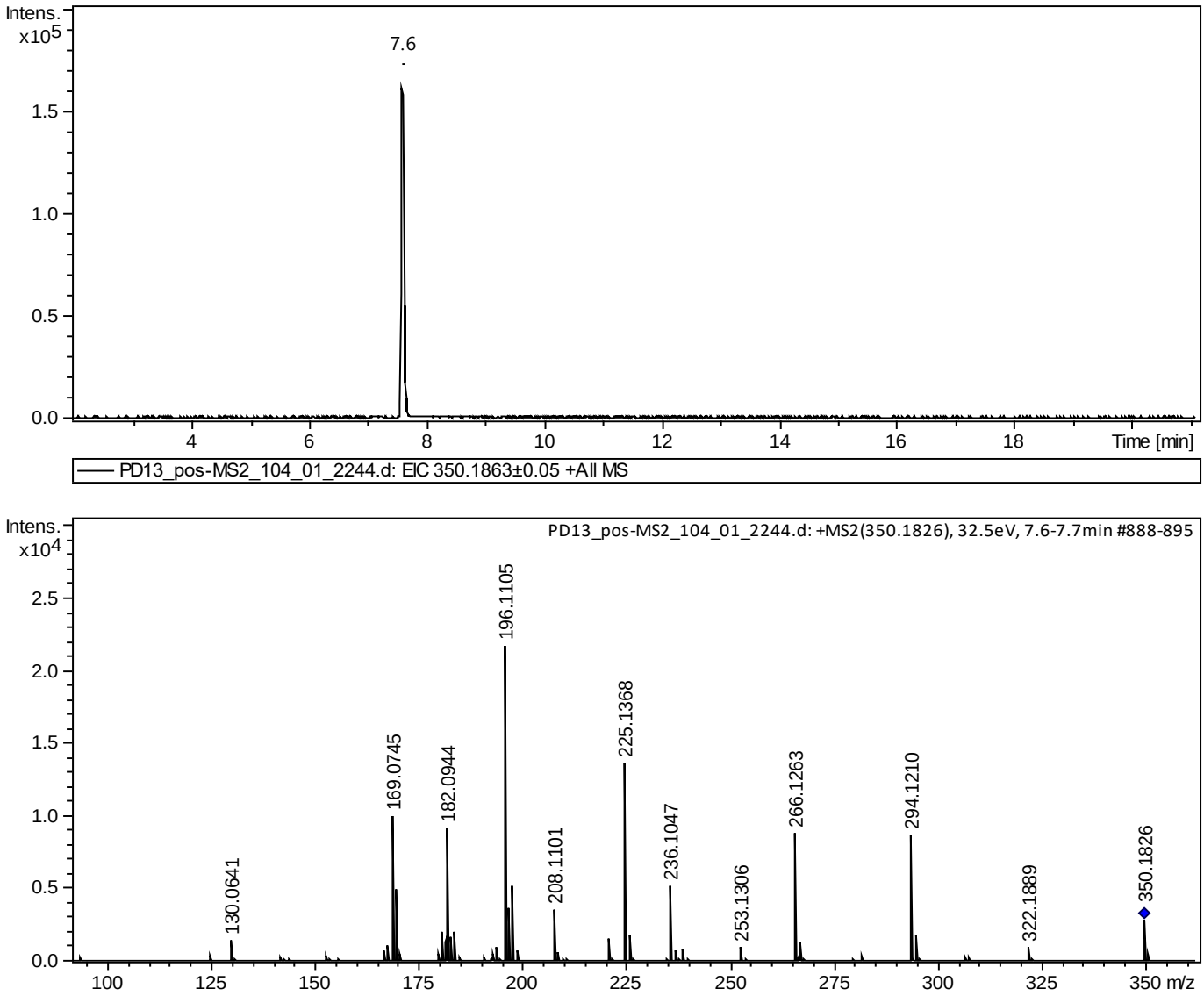

Figure S38. HPLC MS retention time and MS/MS of deoxydihydroisoaustamide (**9**) (reference compound)

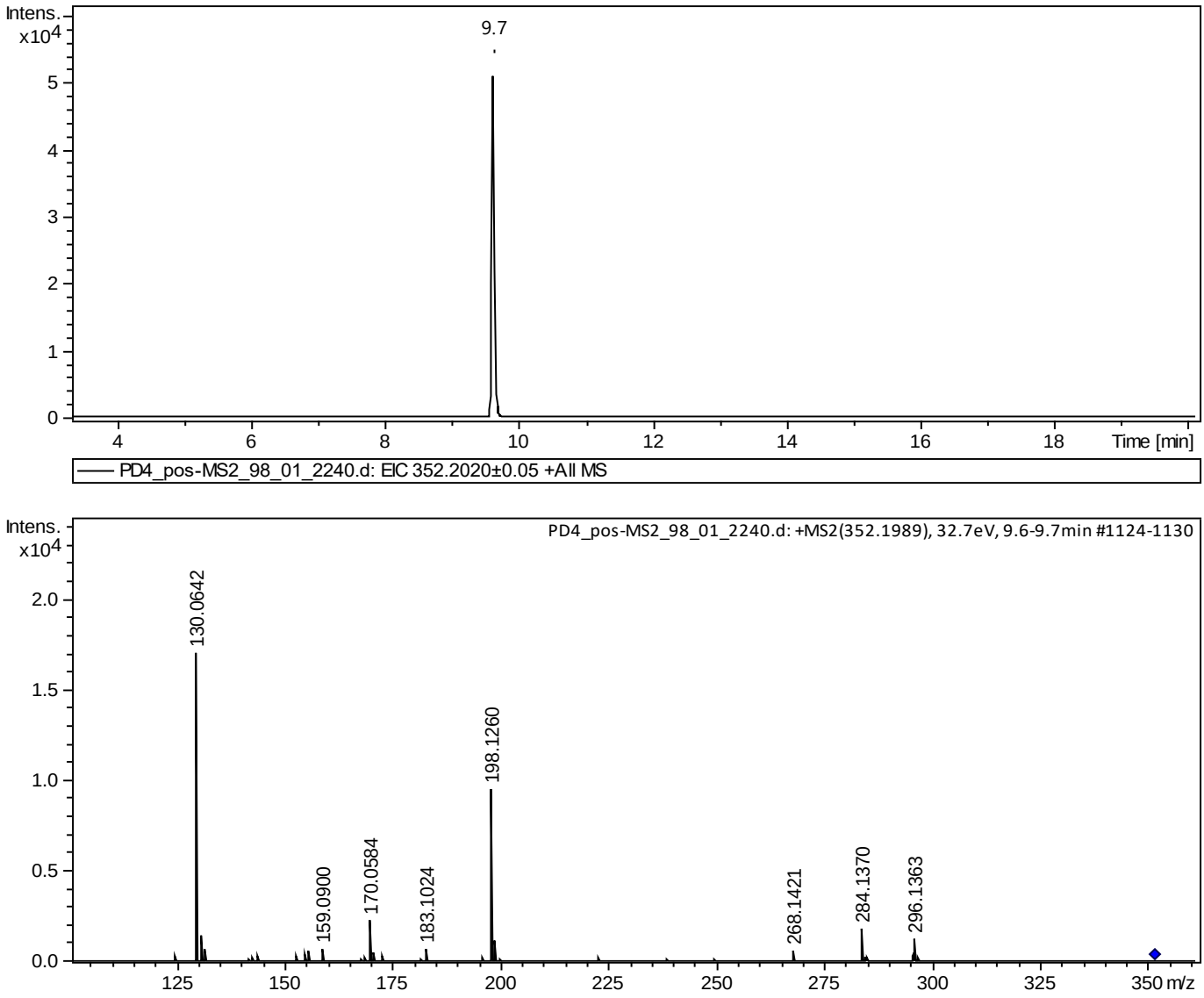

Figure S39. HPLC MS retention time and MS/MS of desoxybrevianamide E (**10**) (reference compound)