Plankton hitch-hikers on naturalist's instruments as silent intruders of aquatic ecosystems: current risks and possible prevention

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Supplementary Material 1

Detailed methodology of toxicological analyses performed for the purpose of this study and the results in the graphical form. Abbreviations: APs – anabaenopeptins, ATX-a – anatoxin-a, BMAA – β-N-methylamino-L-alanine, CYN – cylindrospermopsin, MCs –microcystins, NOD – nodularin, STXs – saxitoxins

Enzyme-Linked Immunosorbent Assay (ELISA) screening tests

Concentrated cyanobacterial biomass of each strain at a volume of 2 ml was homogenized by ultrasonication for 15 minutes using bath sonicator (Sonorex RK156, Banelin electronics, Berlin, Germany) and subsequently for 1 minute using ultrasonic probe MS 73 microtip and HD 2070 homogenizer; Bandelin electronics, Berlin, Germany). Extracts were centrifuged for 10 minutes at 15,000 rpm using microcentrifuge type 157 (Ole Dich Instrumentmakers APS, Hvidovre, Denmark). Supernatants were filtered through a GHP Acrodisc 13 mm syringe filters with 0.2 µm GHP membrane and a minispike outlet (PALL Corporation, New York, NY, USA). Filtrates were analysed for APs, ATX-a, BMAA and STXs using ELISA kits and manufacturer protocols (Eurofins Abraxis, Warminster, PA, US; Product No. 520070, 520060, 520040, 52255B, respectively).

Sample preparation for chromatographic analyses

Samples were analysed for the presence of CYN, MCs and NOD. Dense cyanobacterial biomass of each strain at a volume 30-100 ml was filtered through GF/C filters (diameter 47 mm) using a glass vacuum filtration kit. Subsequently, the filters were freeze-dried using freeze dryer (Alpha 1-4 LSCplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and extracted in 75% methanol by ultrasonication for 15 minutes using bath sonicator (Sonorex RK156, Banelin electronics, Berlin, Germany) and then 1 min. using ultrasonic probe MS 73 microtip and HD 2070 homogenizer; Bandelin electronics, Berlin, Germany). The obtained extracts were centrifuged for 10 min. at 15,000 rpm using microcentrifuge type 157 (Ole Dich Instrumentmakers APS, Hvidovre, Denmark). Collected supernatants were then evaporated to dryness using nitrogen gas (>99.99%) and a temperature of 50 °C. Dried extracts were reconstituted in either 150 µl of 75% methanol (MCs and NOD analyses) and 150 µl of Milli-Q water (CYN analyses) and then filtered through a GHP Acrodisc 13 mm syringe filters with 0.2 µm GHP membrane and a minispike outlet (PALL Corporation, New York, NY, USA). Such prepared samples were kept at -20°C prior to chromatographic analyses.

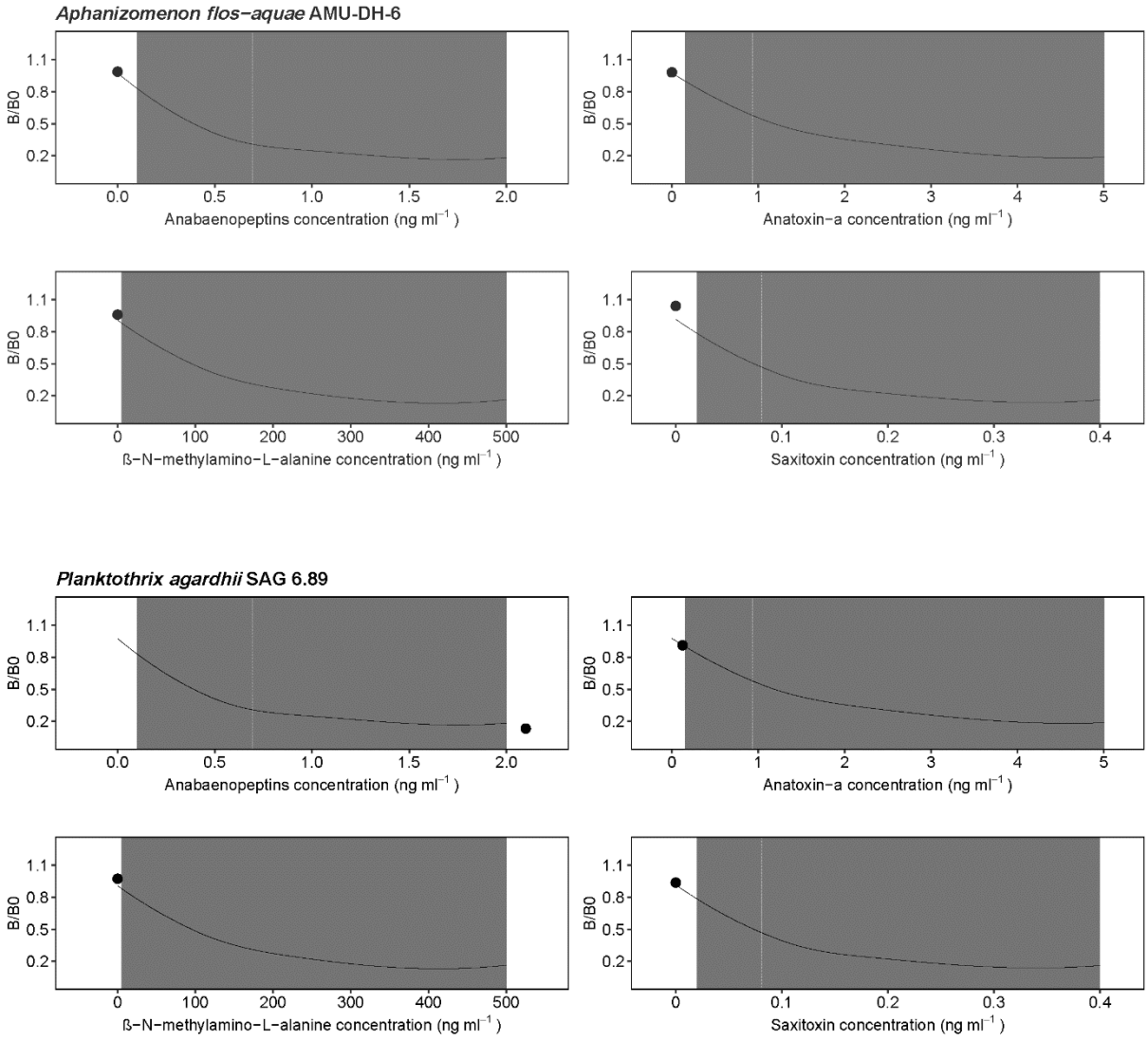
Chromatographic analyses for the presence CYN, MCs and NOD in the extracted samples

The HPLC-DAD was conducted using the Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany). The LC-MS was performed using the Agilent 1200 Rapid Resolution LC coupled to a Bruker Daltonics HCT ultra ion trap mass spectrometer with electrospray ion ESI source. The analyses were performed at Biochemistry, Faculty of Science and Engineering, Åbo Akademi University in Turku (Finland) according to Meriluoto and Spoof (2005), Kokociński et al. (2013), Hautala et al. (2013). HPLC-DAD chromatogram analyses involved identification of CYN, MCs variants, and NOD by comparing retention times and absorption spectra of the compounds to the

authentic standards using the ChemStation for LC 3D systems software (Agilent Technologies, Waldbronn, Germany). Mass spectrometric analyses were based on the comparison of the retention times and the observed m/z values of the compounds in relation to the authentic standards using the ESI Compass 1.3 for HCT/esquire software (Bruker Daltonik GmbH, Bremen, Germany).

The results of ELISA tests

The black curve is loess-fitted standard curve constructed based on the standards provided by the manufacturer. Black circle indicates the concentration of a given toxin. Detection limit is demonstrated by the grey area. White vertical line indicates the concentration of the cyanotoxin in positive control that should be 0.75 ng ml^{-1} (APs), 0.75 ng ml^{-1} (ATX-a), 0.075 ng ml^{-1} (STXs). B is the absorbance of a given standard containing toxin, while B0 is the absorbance value for standard zero. B/B0 was calculated for each standard for the purpose of standard curve preparation. Data on the presence of APs, ATX-a, BMAA and STXs in *A. gracile* AMU-DH-1 and *R. raciborskii* SAG 1.97 have been previously published (Falfushynska et al. 2021).



References:

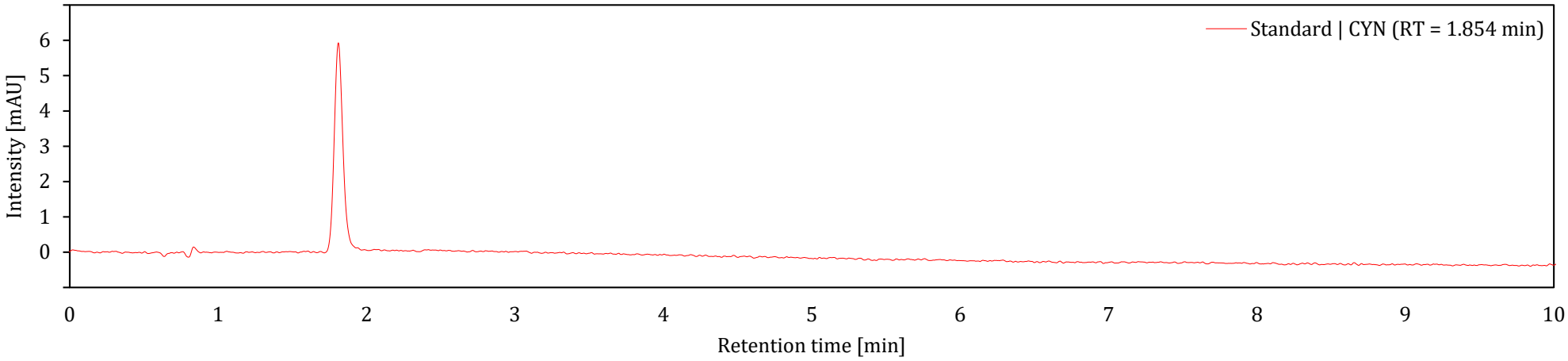
Falfushynska H, Horyn O, Osypenko I, Rzymski P, Wejnerowski Ł, Dziuba MK, Sokolova IM (2021) Multibiomarker-based assessment of toxicity of central European strains of filamentous cyanobacteria *Aphanizomenon gracile* and *Raphidiopsis raciborskii* to zebrafish *Danio rerio*. Water Res. 194, 116923. https://doi.org/10.1016/j.watres.2021.116923

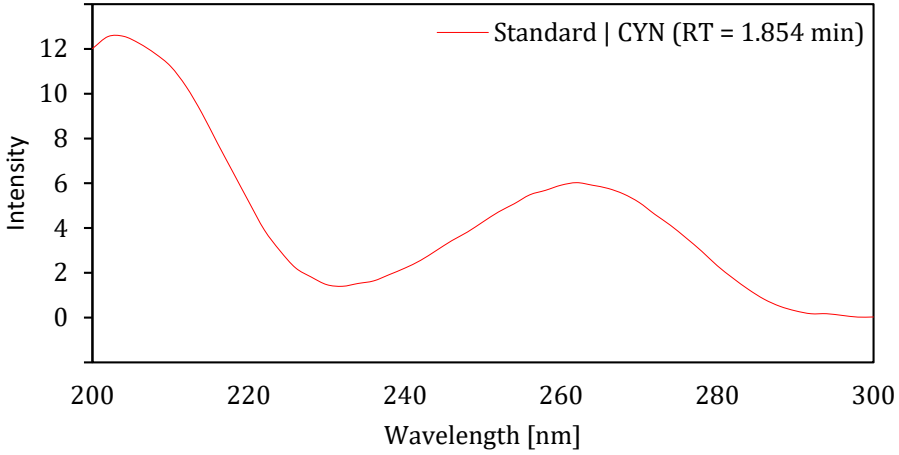
Hautala H, Lemminmäki U, Spoof L, Nybom S, Meriluoto J, Vehniäinen M (2013) Quantitative PCR detection and improved sample preparation of microcystin-producing *Anabaena*, *Microcystis* and *Planktothrix*. Ecotox. Envrion. Saf. 87, 49–56. https://doi.org/10.1016/j.ecoenv.2012.10.008

Kokociński M, Mankiewicz-Boczek J, Jurczak T, Spoof L, Meriluoto J, Rejmonczyk E, Hautala H, Vehniäinen M, Pawełczyk J, Soininen J (2013) *Aphanizomenon gracile* (Nostocales), a cylindrospermopsin-producing cyanobacterium in Polish lakes. Environ. Sci. Pollut. Res. 20, 5243–5264. https://doi.org/10.1007/s11356-012-1426-7

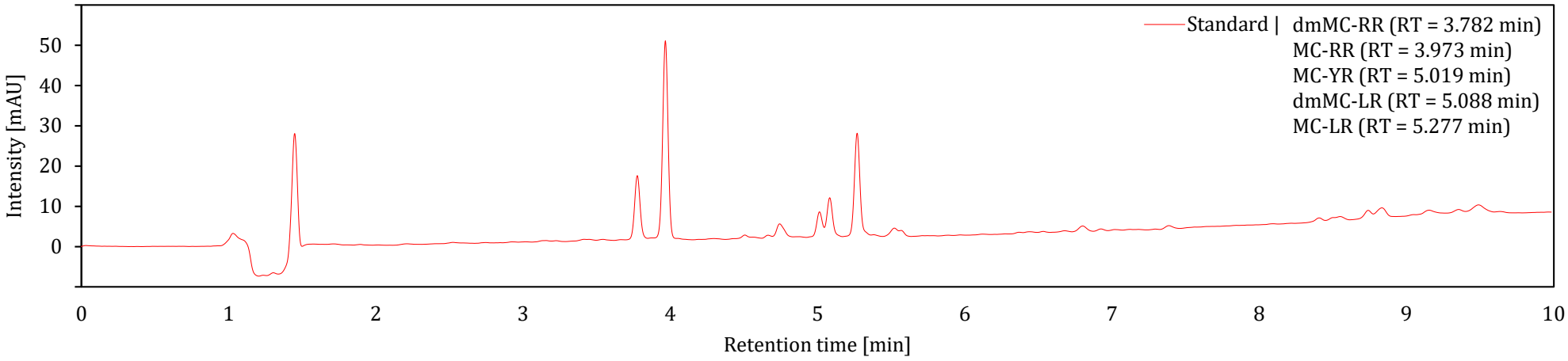
Meriluoto J, Spoof L (2005) SOP: Extraction of microcystins in biomass filtered on glass-fibre filters or in freeze-dried cyanobacterial biomass. In: Meriluoto, K., Codd, G.A. (Eds.), Toxic: cyanobacterial monitoring and cyanotoxin analysis. Åbo Akademi University Press, Åbo, Finland.

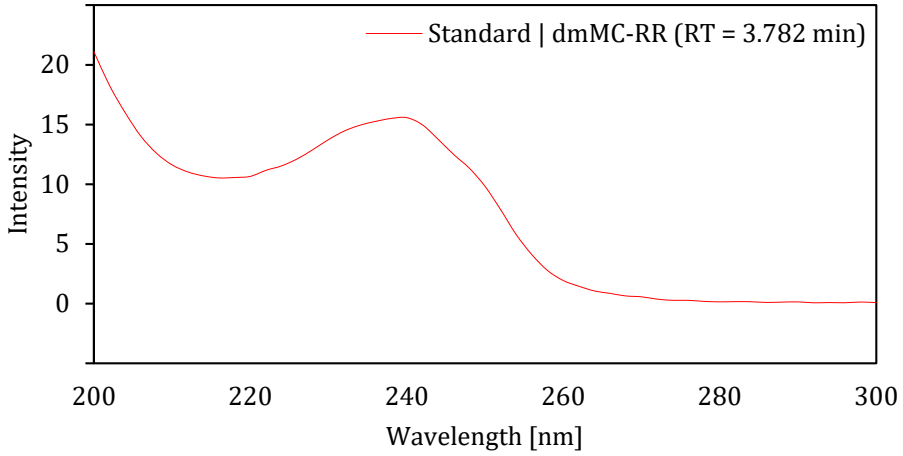
The results of HPLC-DAD analysis | Chromatogram of CYN standard and CYN spectrum

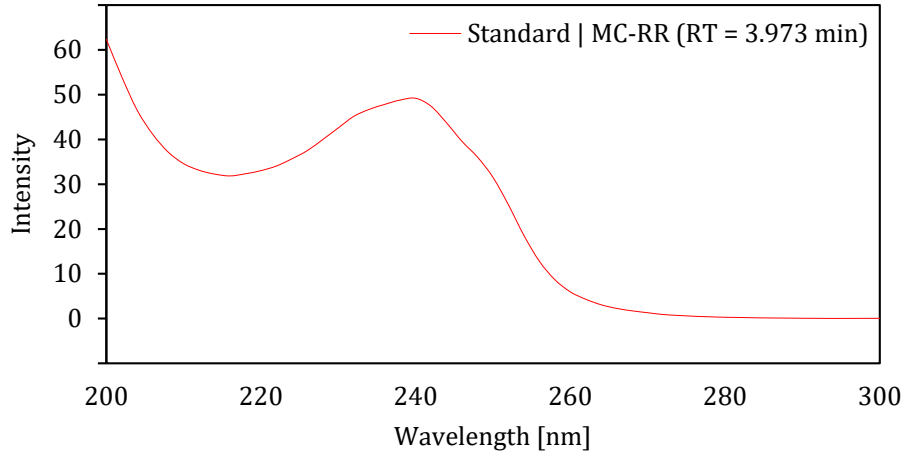




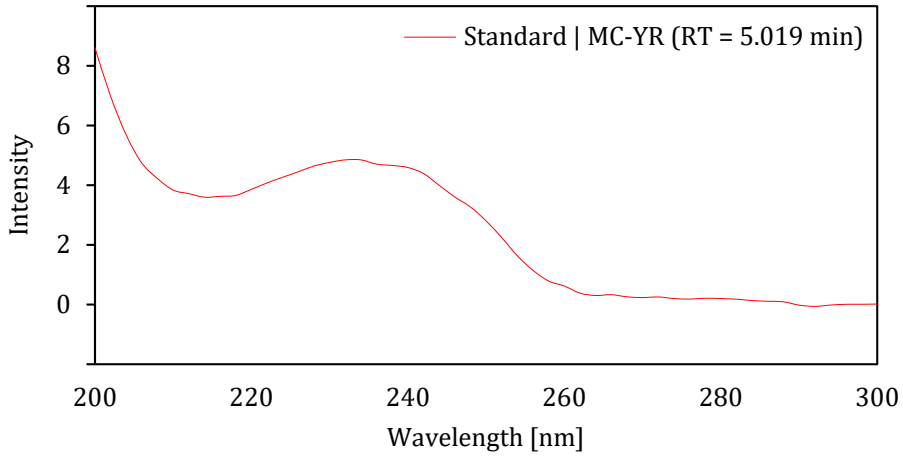
The results of HPLC-DAD analysis | Chromatogram of MCs standard (NIES107) and MCs spectra

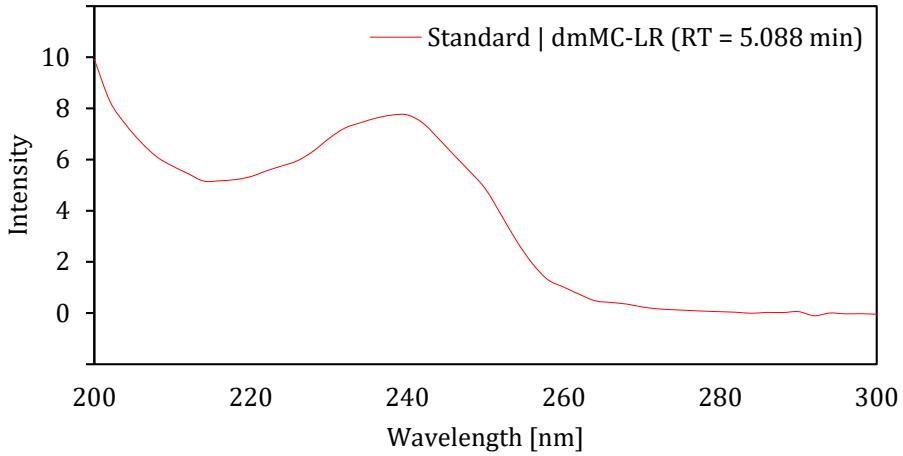


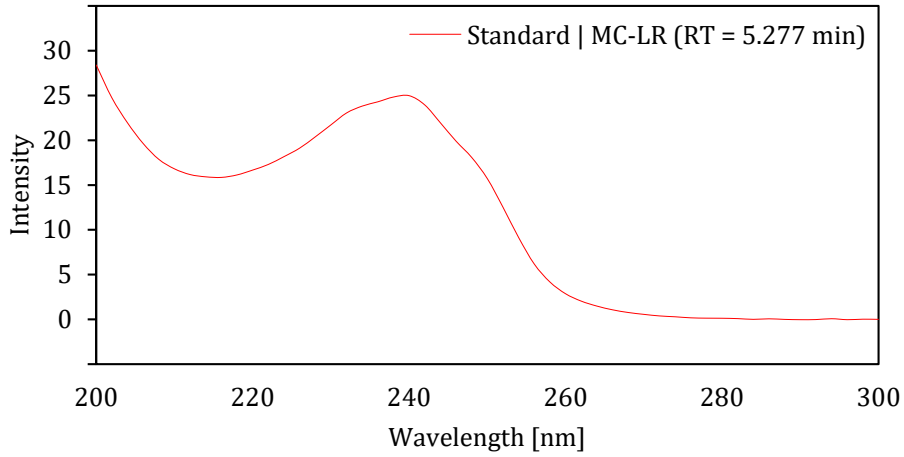




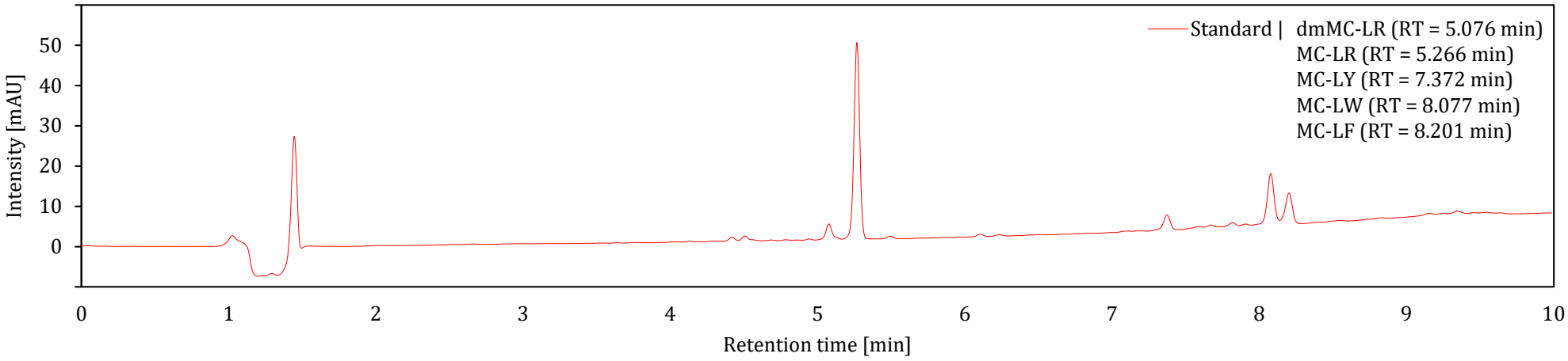
The results of HPLC-DAD analysis | MCs spectra in the standard (NIES107)

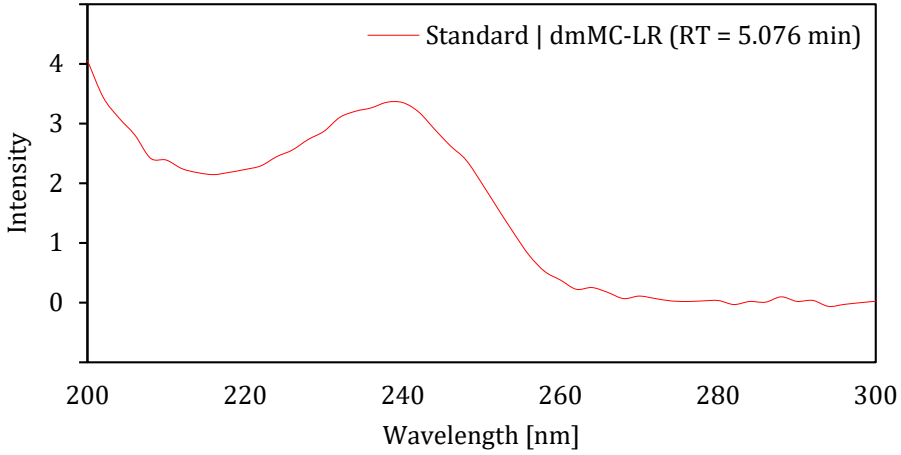


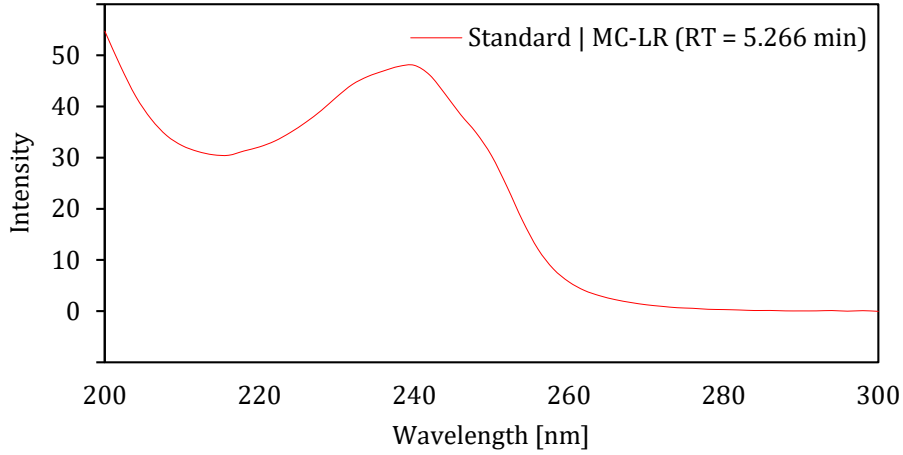




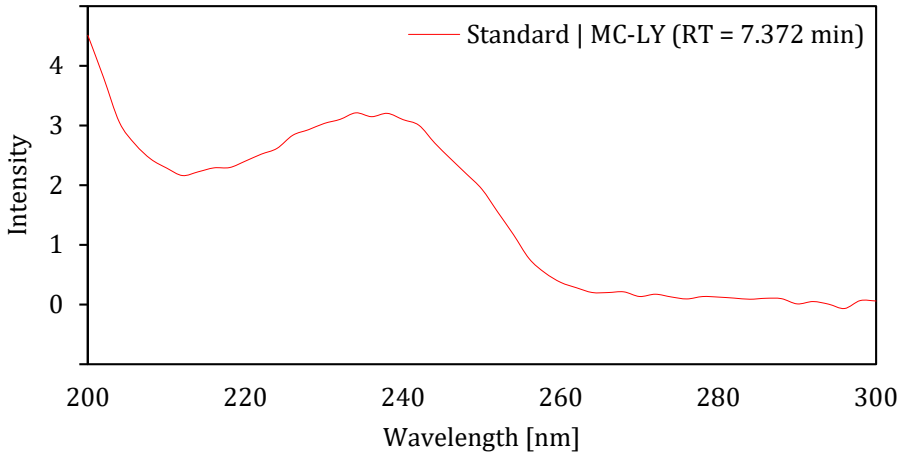
The results of HPLC-DAD analysis | Chromatogram of MCs standard (PCC8720) and MCs spectra

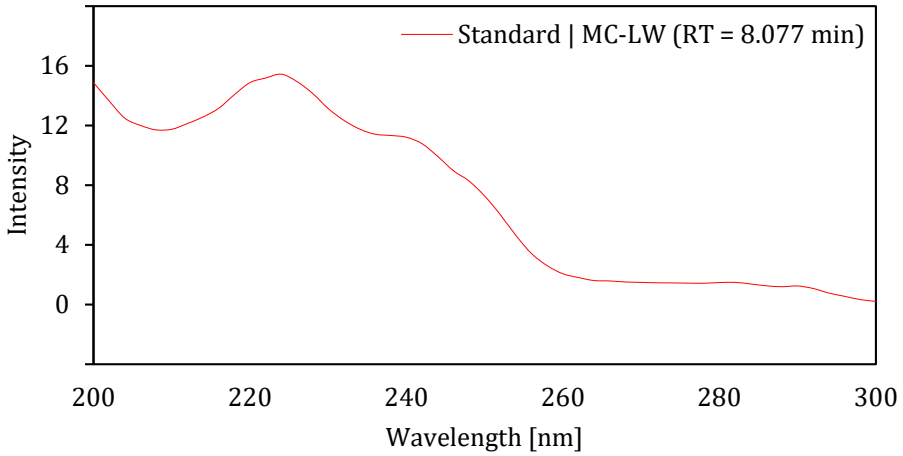


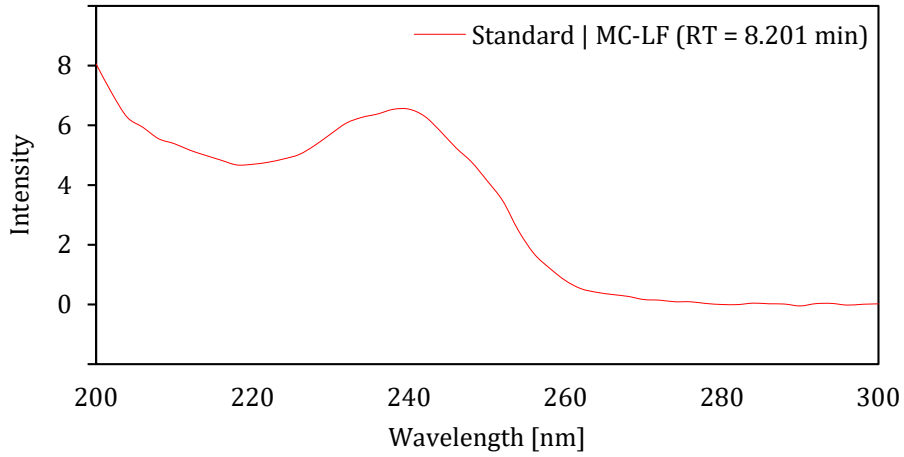




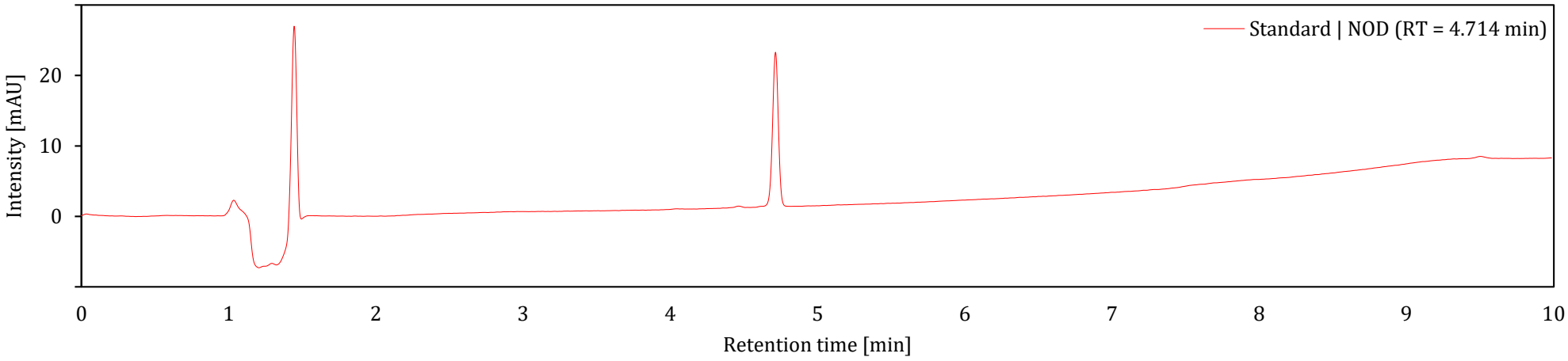
The results of HPLC-DAD analysis | MCs spectra in the standard (PCC8720)

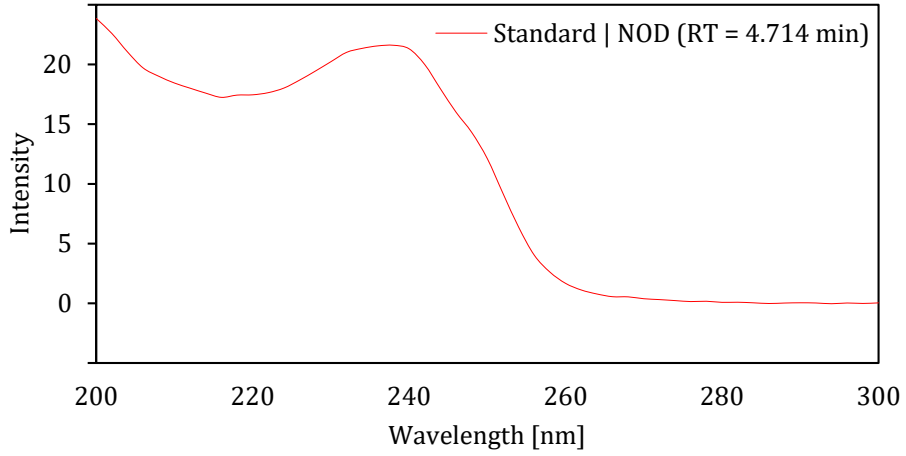




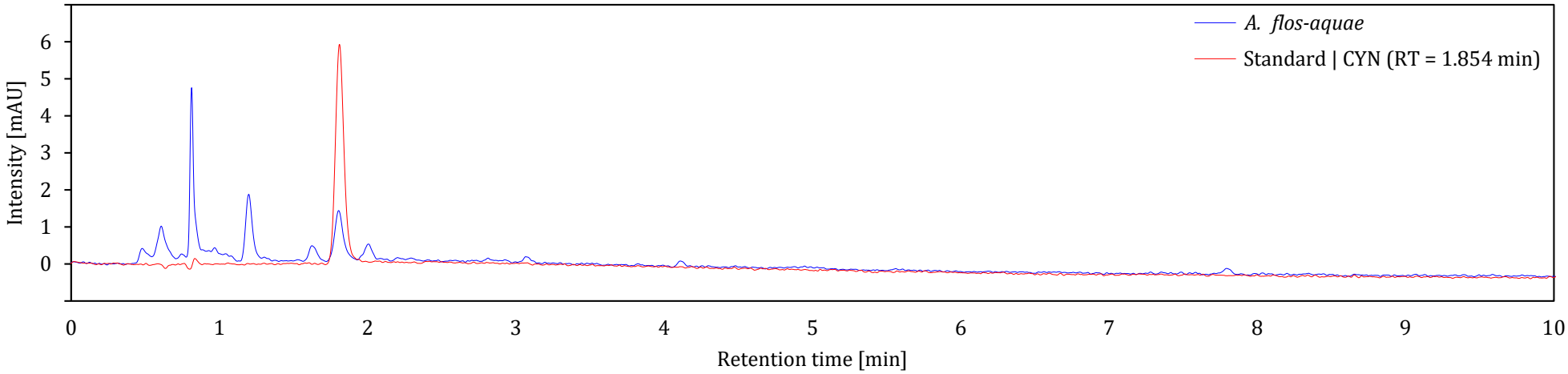


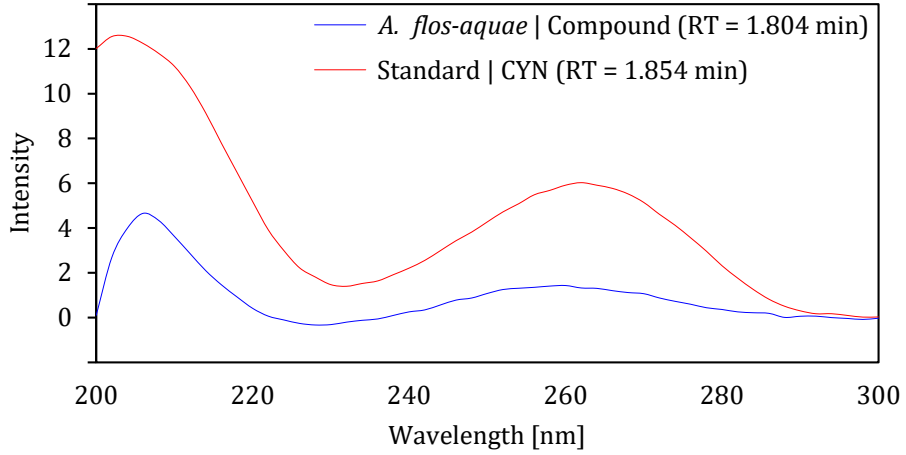
The results of HPLC-DAD analysis | | Chromatogram of NOD standard and NOD spectrum





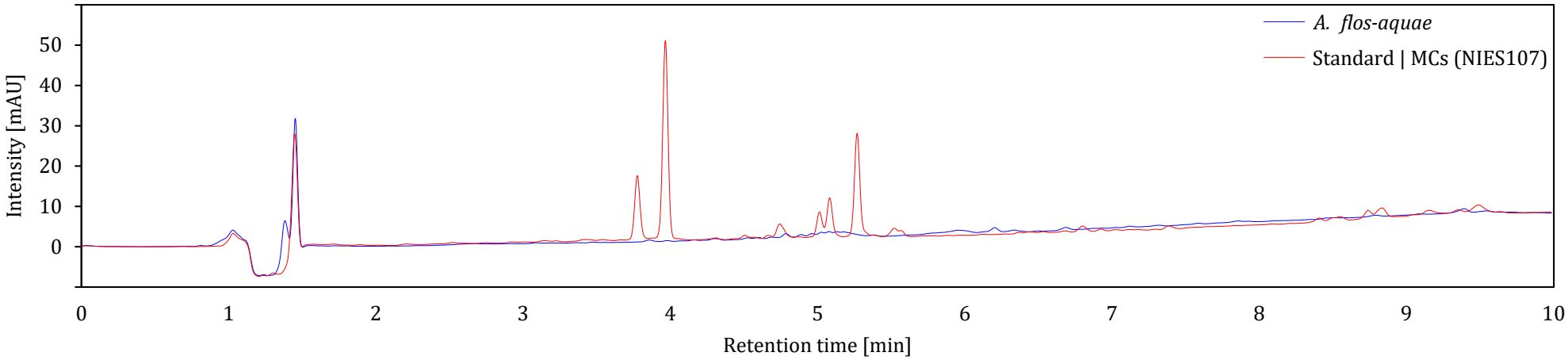
The results of HPLC-DAD analysis | Chromatogram of *Aphanizomenon flos-aquae* AMU-DH-6 and compound spectra

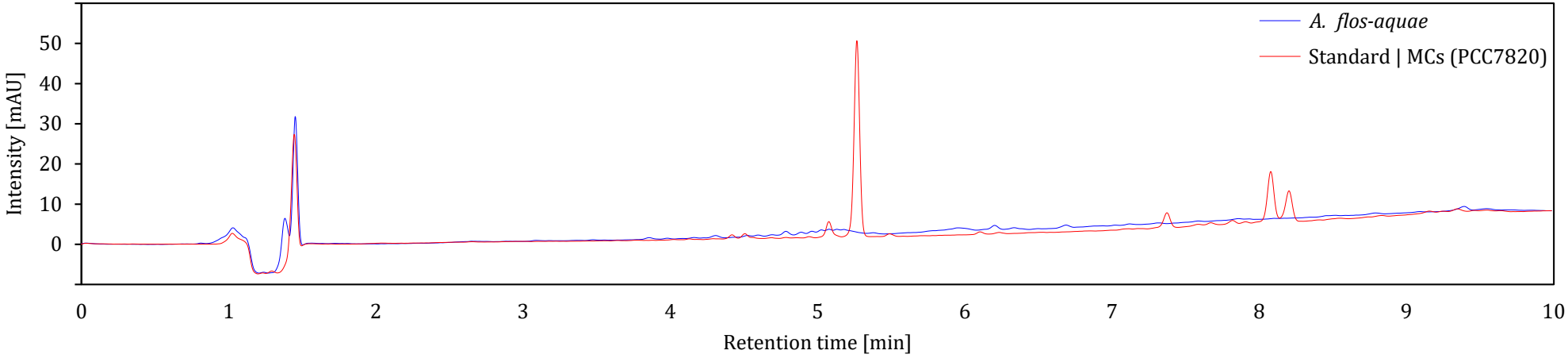




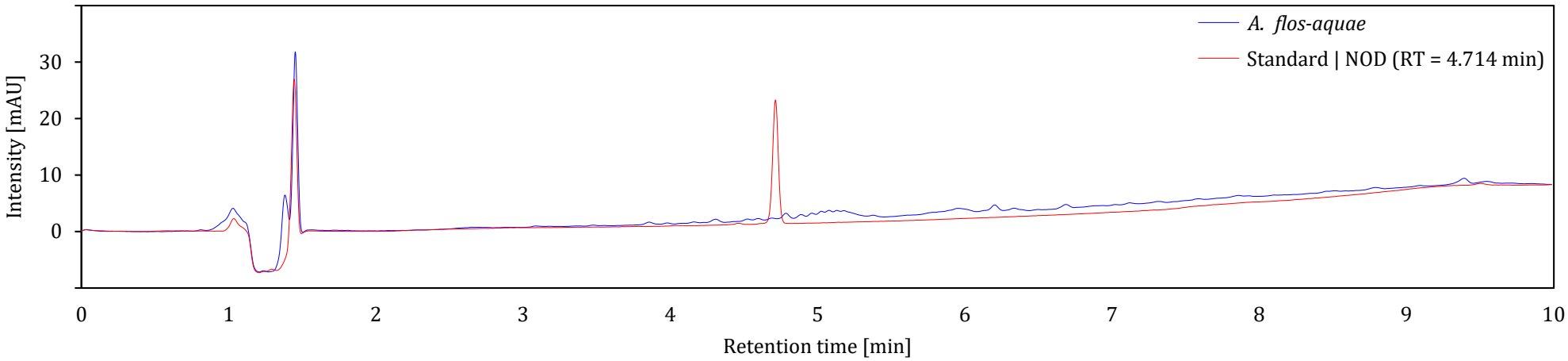
A. flos-aquae compounds of similar RT to CYN had a spectrum that did not match the spectrum of the standard.

The results of HPLC-DAD analysis | Chromatograms of *Aphanizomenon flos-aquae* AMU-DH-6 and MCs standards

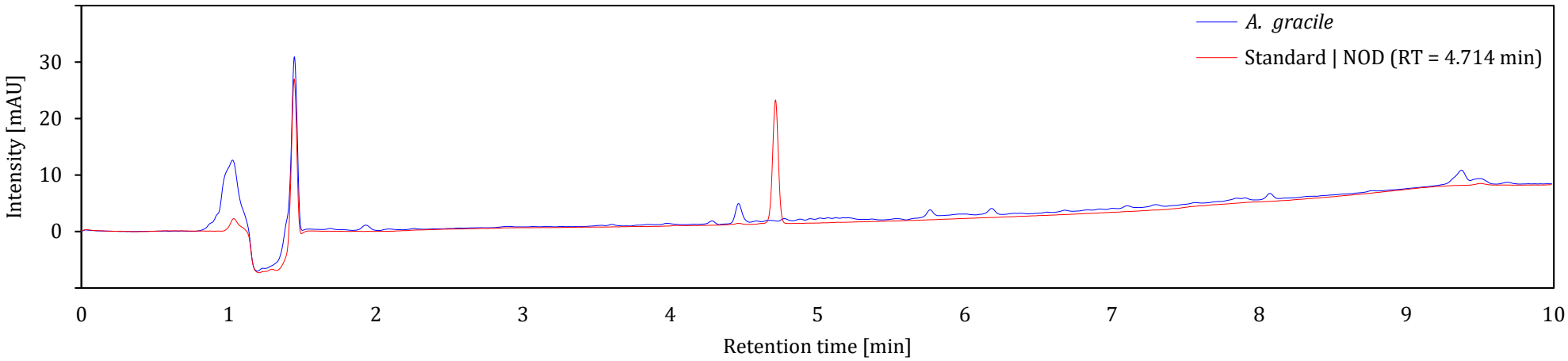




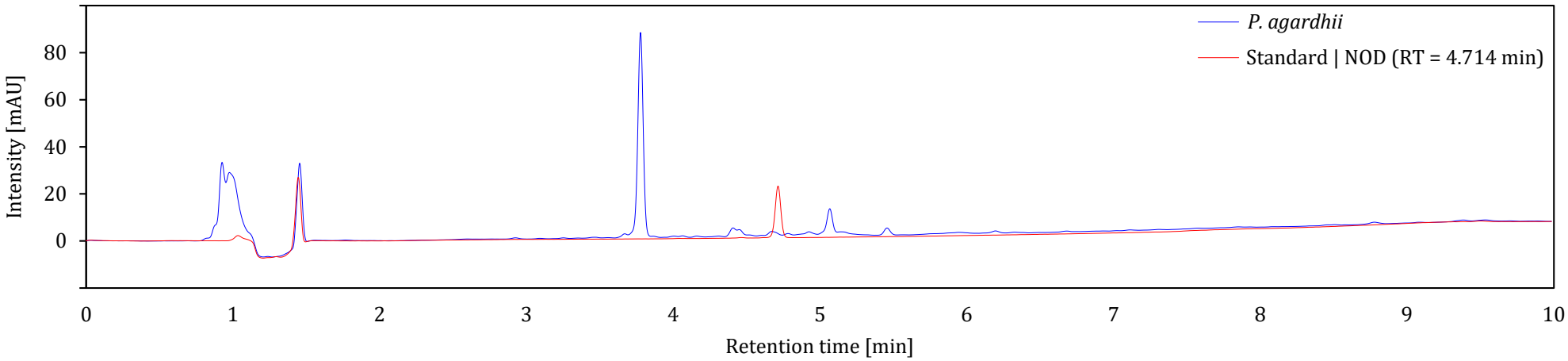
The results of HPLC-DAD analysis | Chromatogram of *Aphanizomenon flos-aquae* AMU-DH-6 and NOD standard



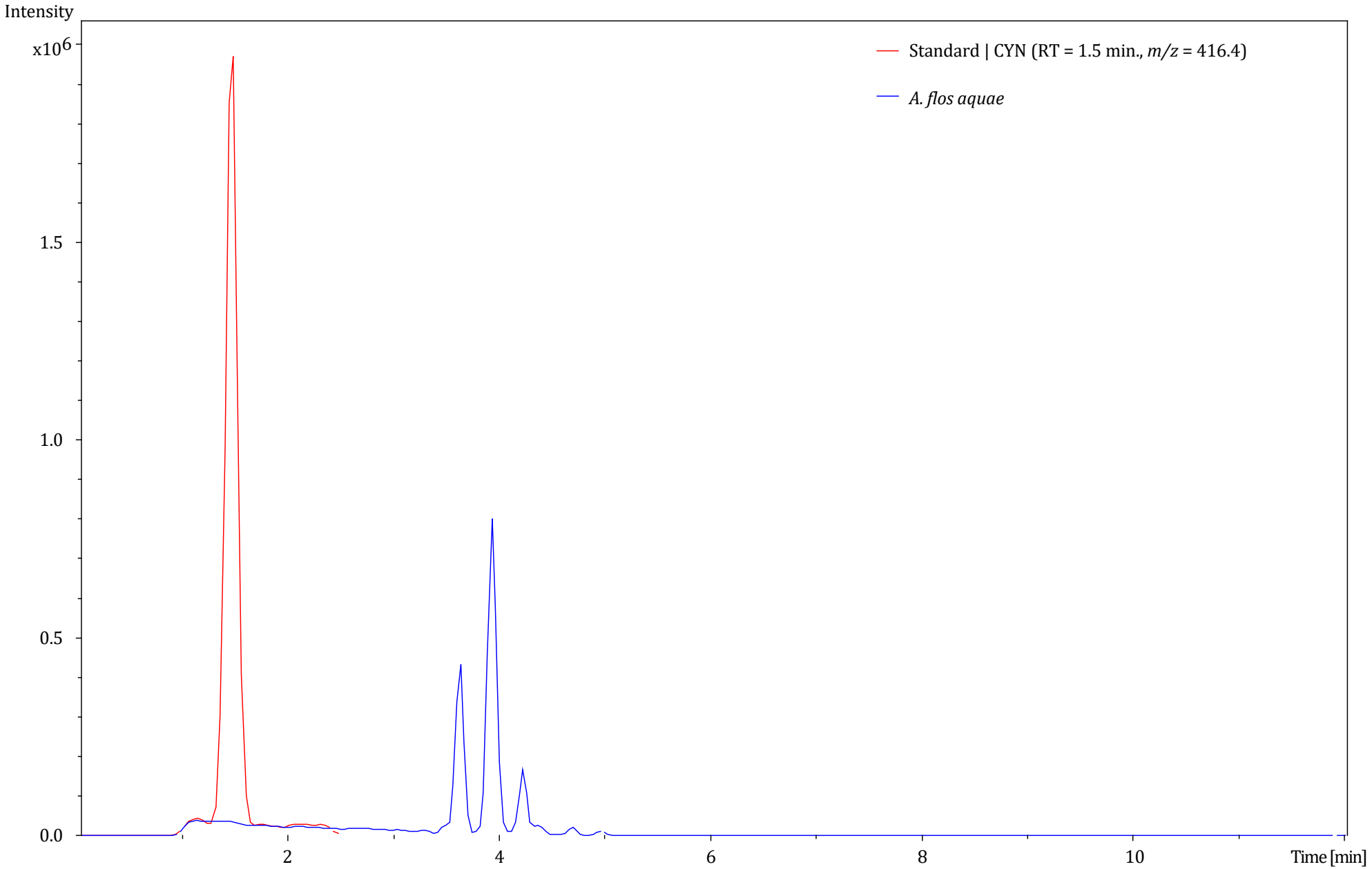
The results of HPLC-DAD analysis | Chromatogram of *Aphanizomenon gracile* AMU-DH-1 and NOD standard



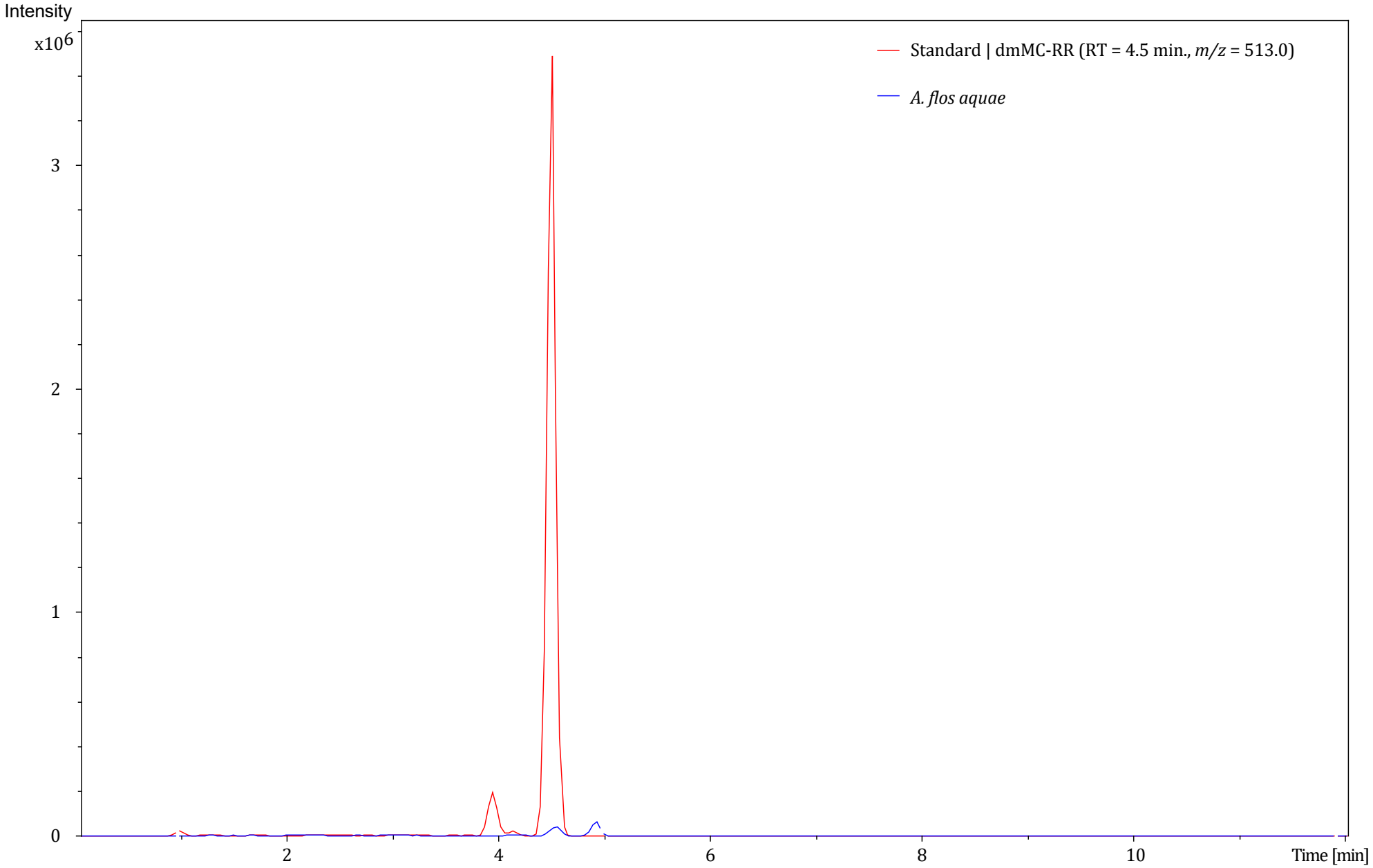
The results of HPLC-DAD analysis | Chromatogram of *Planktothrix agardhii* SAG 6.89 and NOD standard



The results of LC-MS analysis | extracted ion chromatogram of CYN standard and *Aphanizomenon flos-aquae* AMU-DH-6

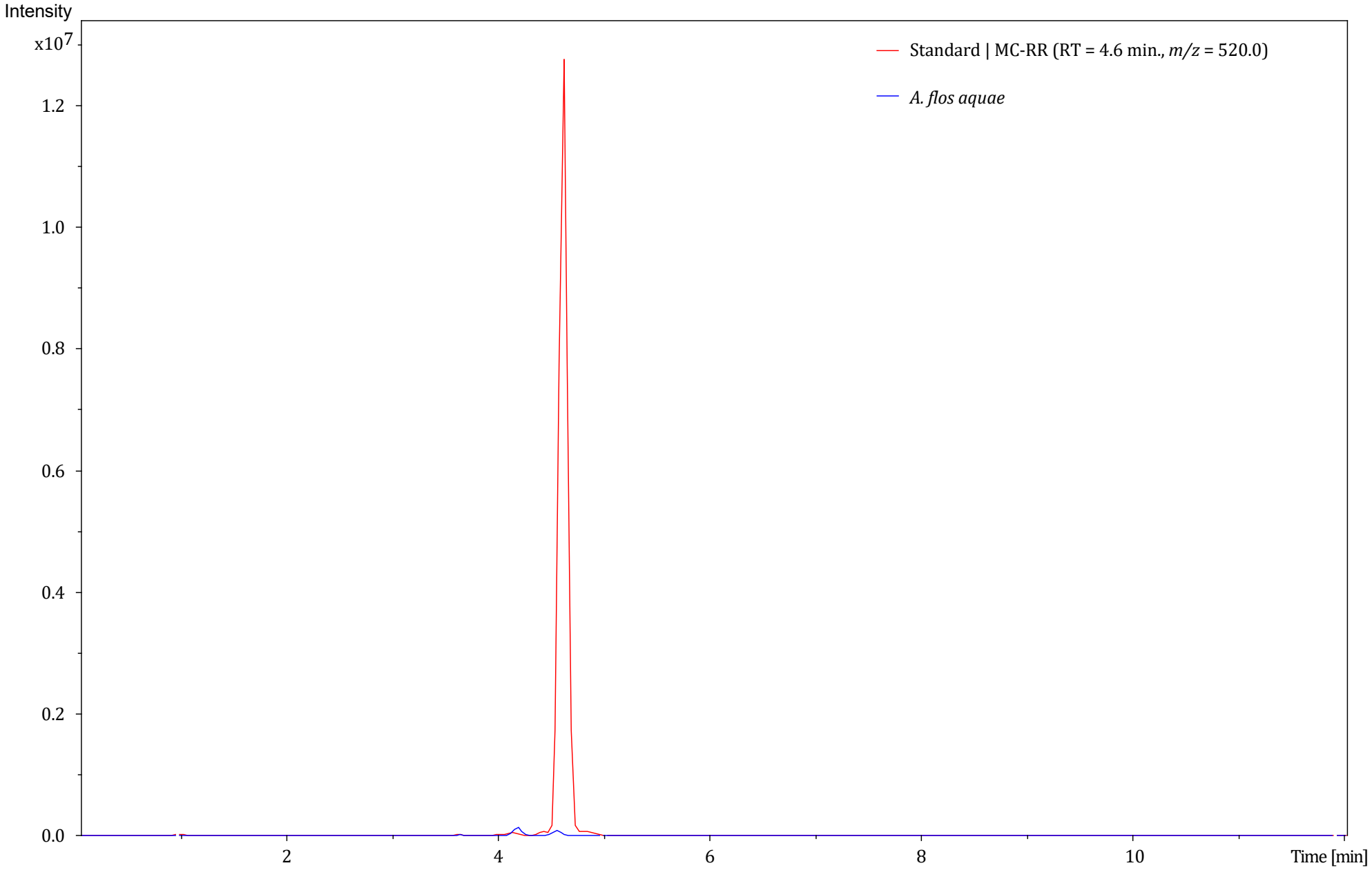


The results of LC-MS analysis | extracted ion chromatogram of dmMC-RR standard (NIES107) and *Aphanizomenon flos-aquae* AMU-DH-6

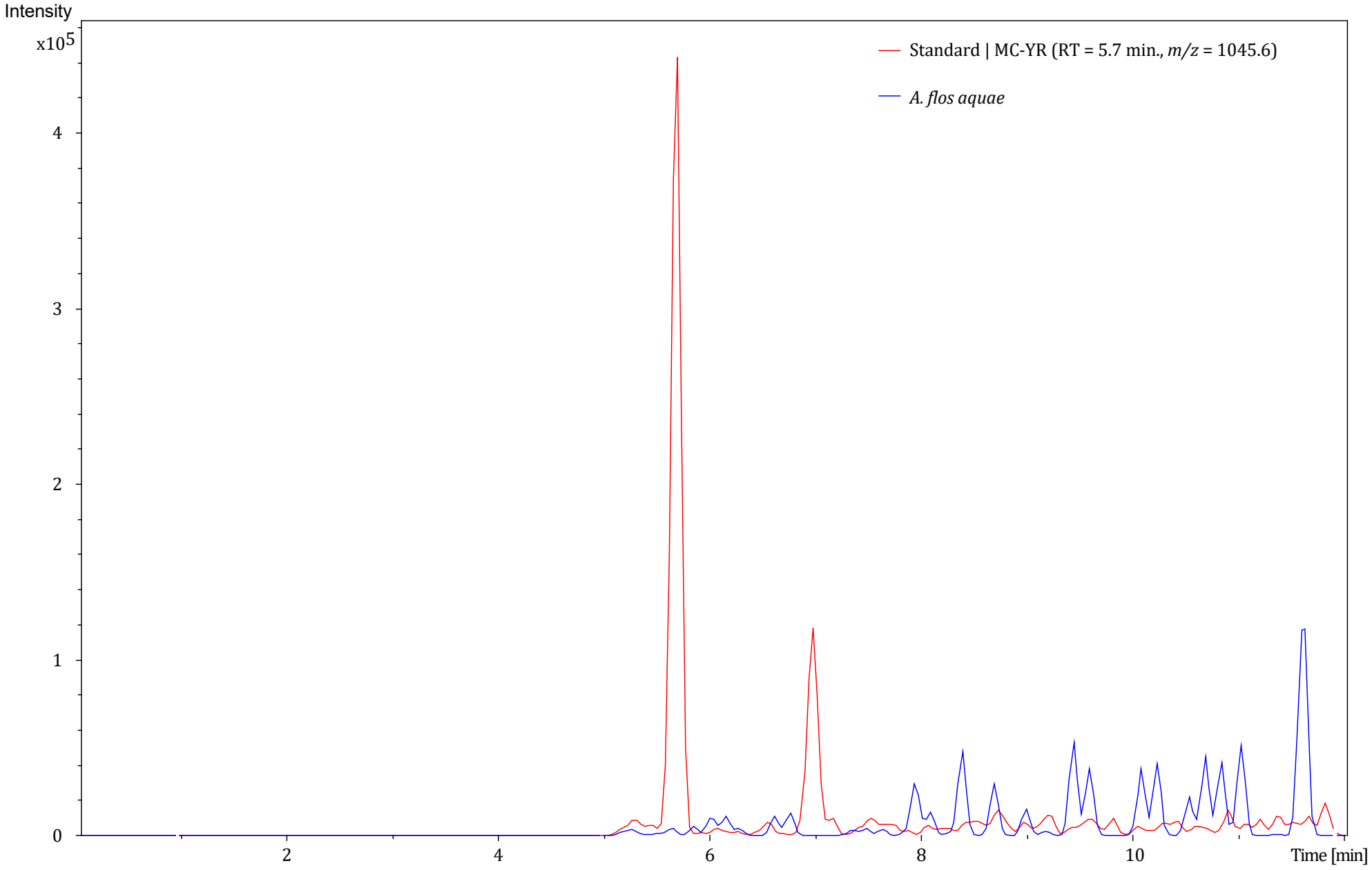


A. flos-aquae compounds of similar RT to dmMC-RR had a spectrum that did not match the spectrum of the standard.

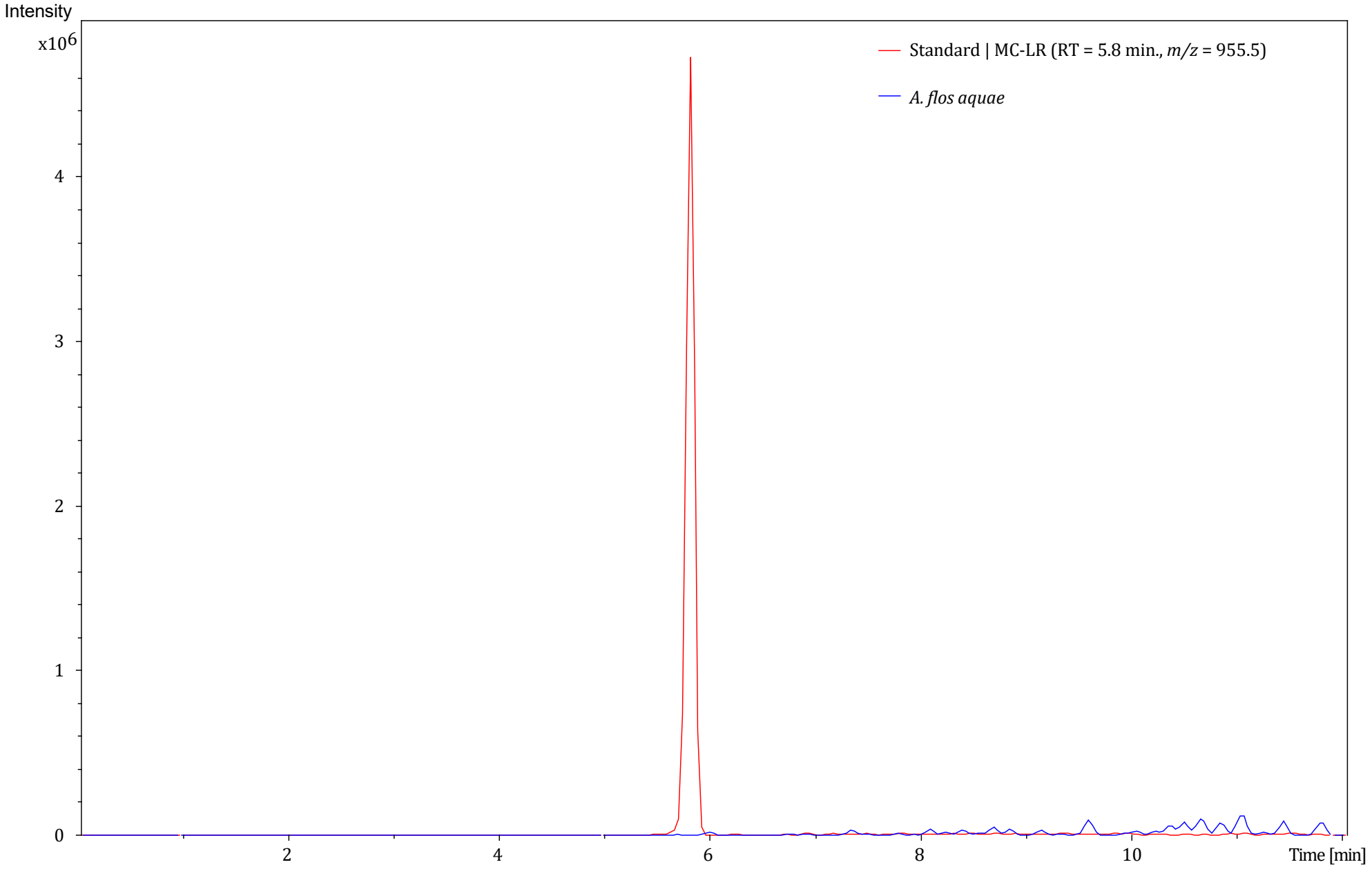
The results of LC-MS analysis | extracted ion chromatogram of MC-RR standard (NIES107) and *Aphanizomenon flos-aquae* AMU-DH-6



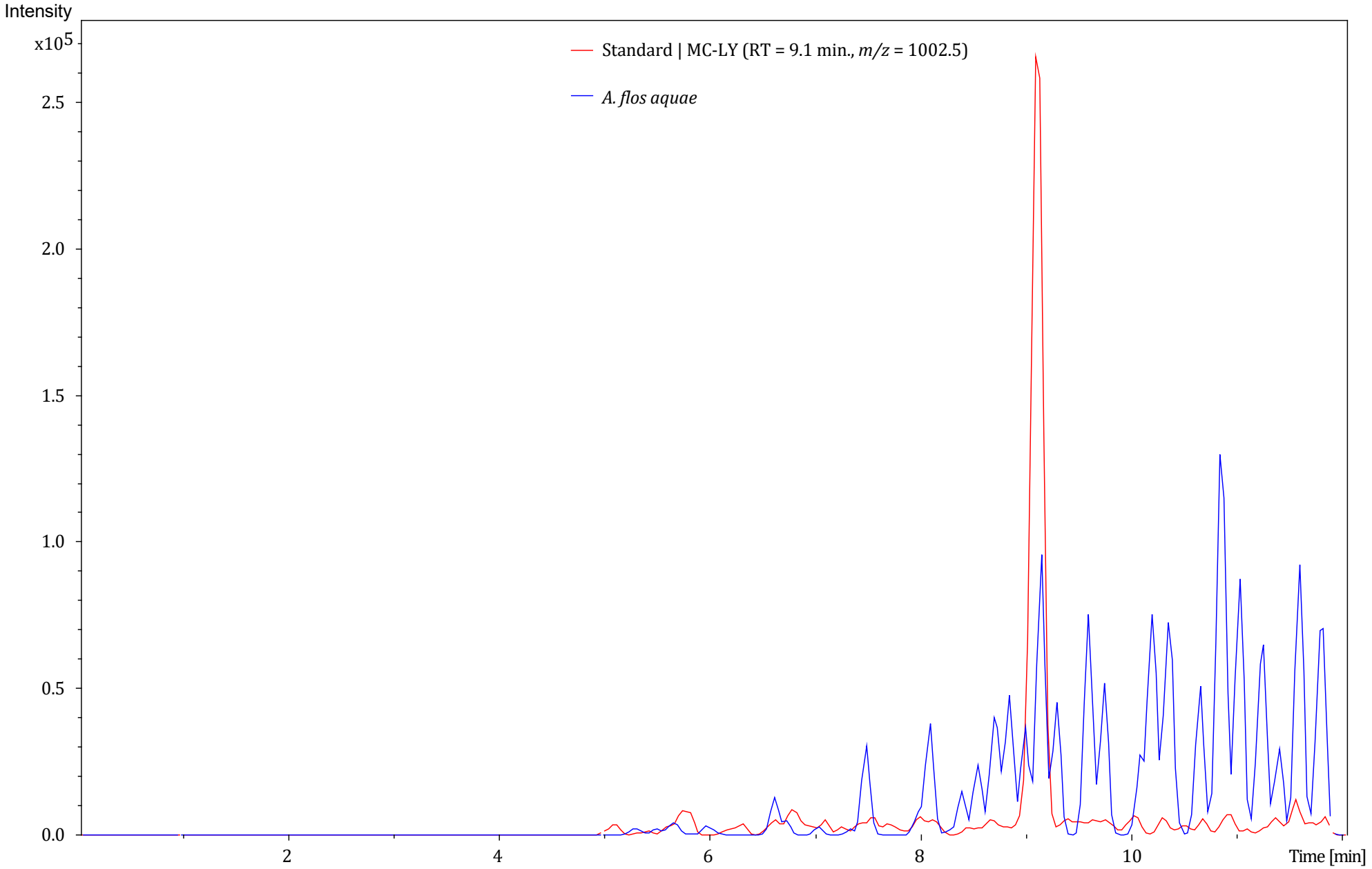
The results of LC-MS analysis | extracted ion chromatogram of MC-YR standard (NIES107) and *Aphanizomenon flos-aquae* AMU-DH-6



The results of LC-MS analysis | extracted ion chromatogram of MC-LR standard (PCC7820) and *Aphanizomenon flos-aquae* AMU-DH-6

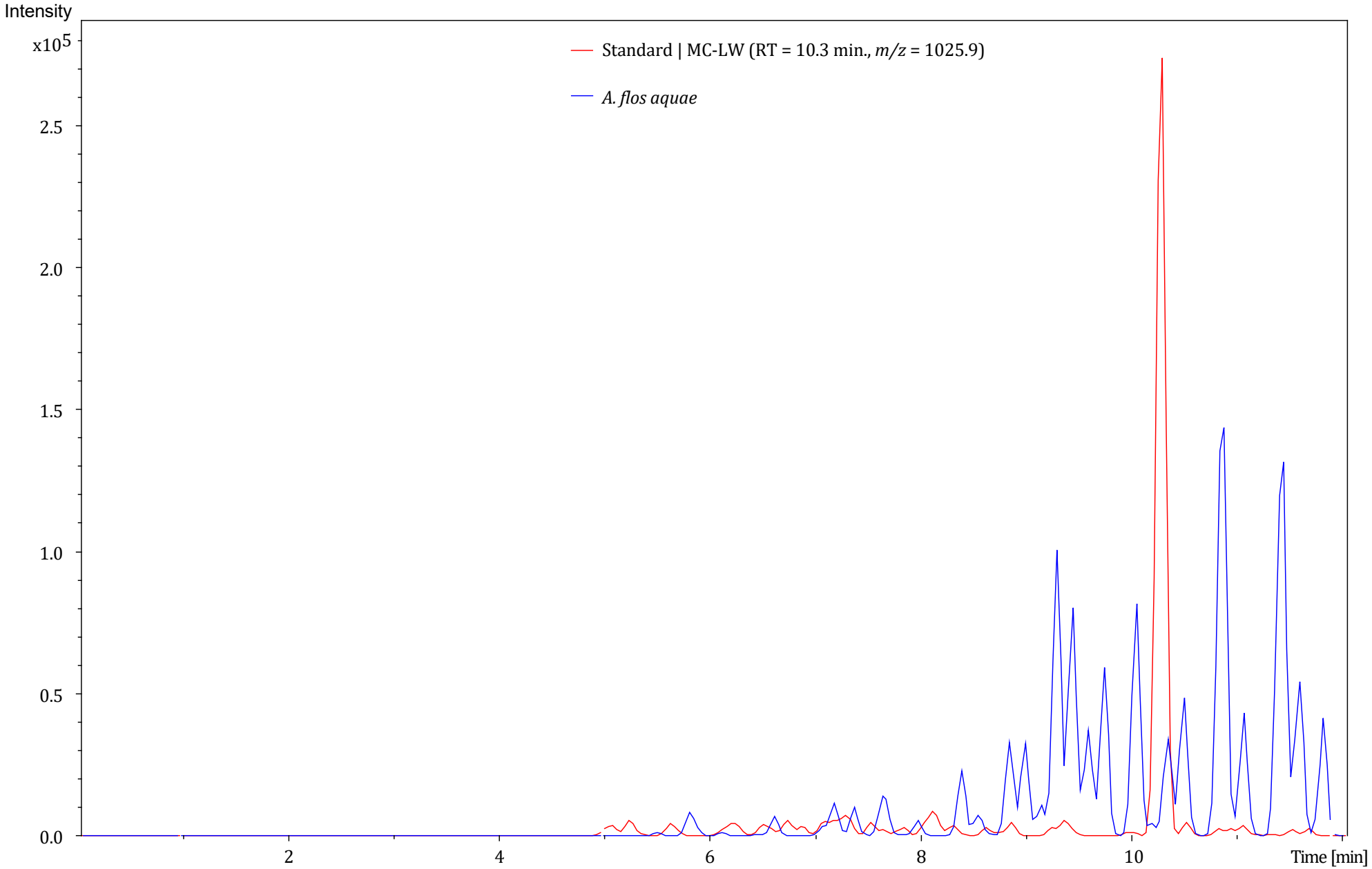


The results of LC-MS analysis | extracted ion chromatogram of MC-LY standard (PCC7820) and *Aphanizomenon flos-aquae* AMU-DH-6



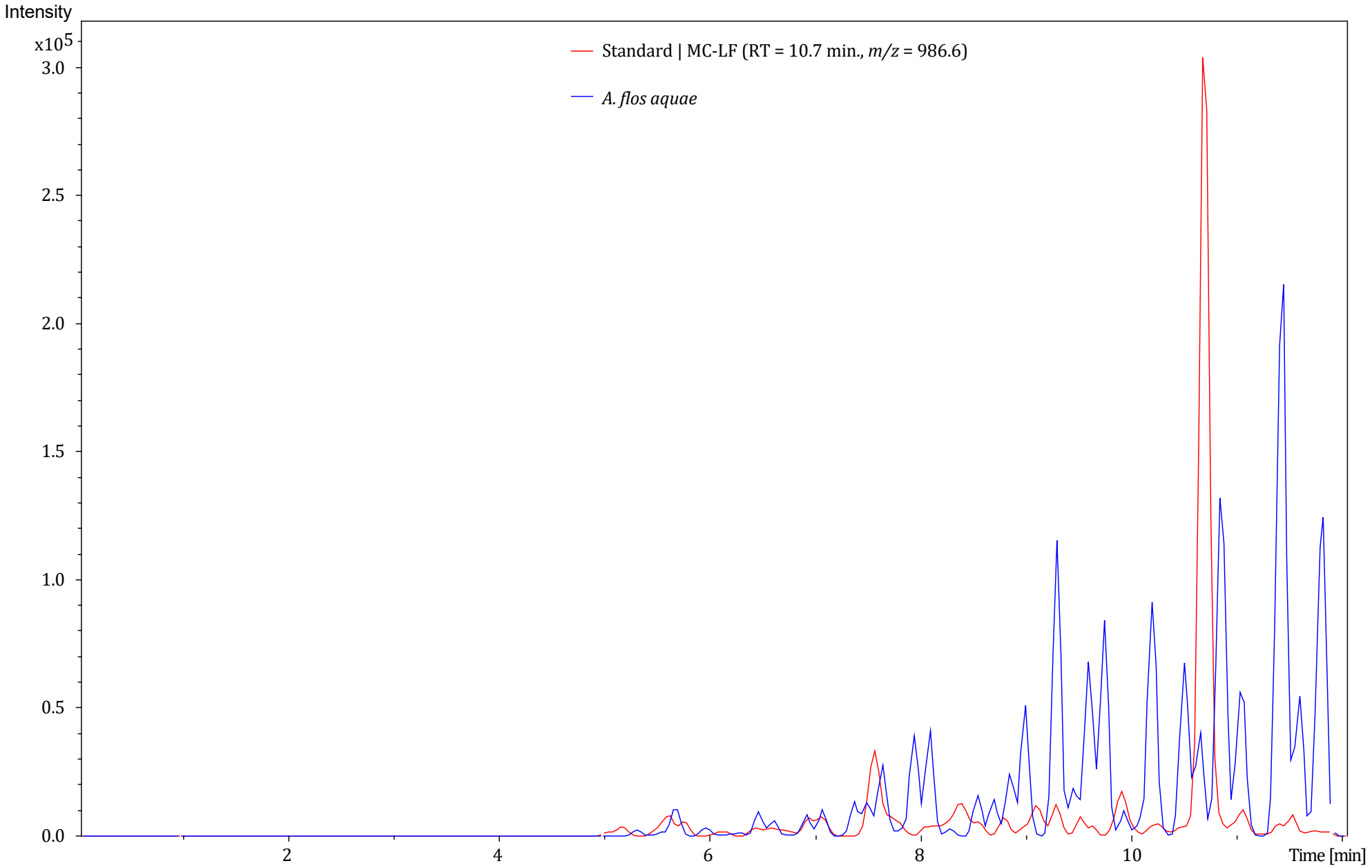
A. flos-aquae compounds of similar RT to MC-LY had a spectrum that did not match the spectrum of the standard.

The results of LC-MS analysis | extracted ion chromatogram of MC-LW standard (PCC7820) and *Aphanizomenon flos-aquae* AMU-DH-6



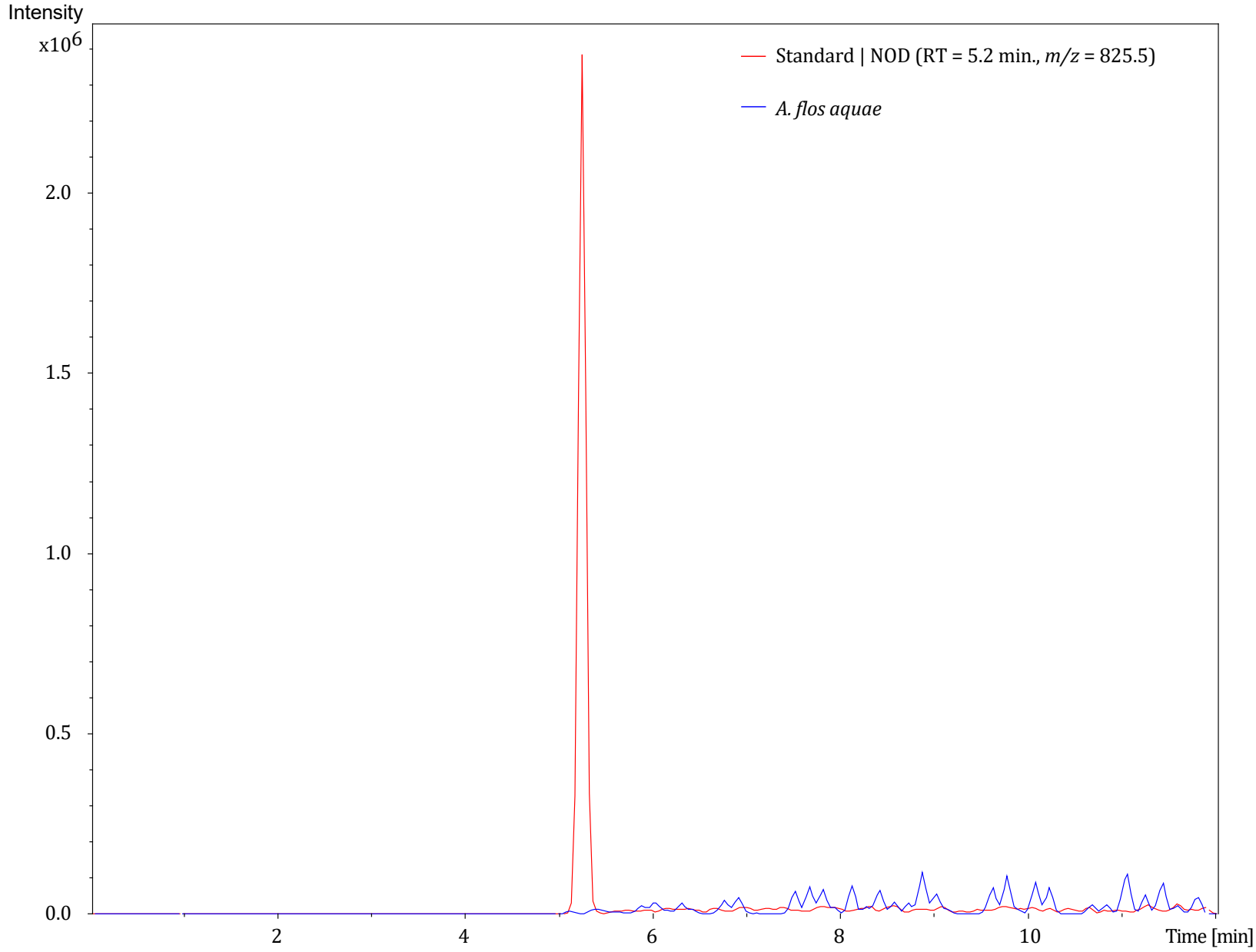
A. flos-aquae compounds of similar RT to MC-LW had a spectrum that did not match the spectrum of the standard.

The results of LC-MS analysis | extracted ion chromatogram of MC-LF standard (PCC7820) and *Aphanizomenon flos-aquae* AMU-DH-6



A. flos-aquae compounds of similar RT to MC-LF had a spectrum that did not match the spectrum of the standard.

The results of LC-MS analysis | extracted ion chromatogram of NOD standard and *Aphanizomenon flos-aquae* AMU-DH-6



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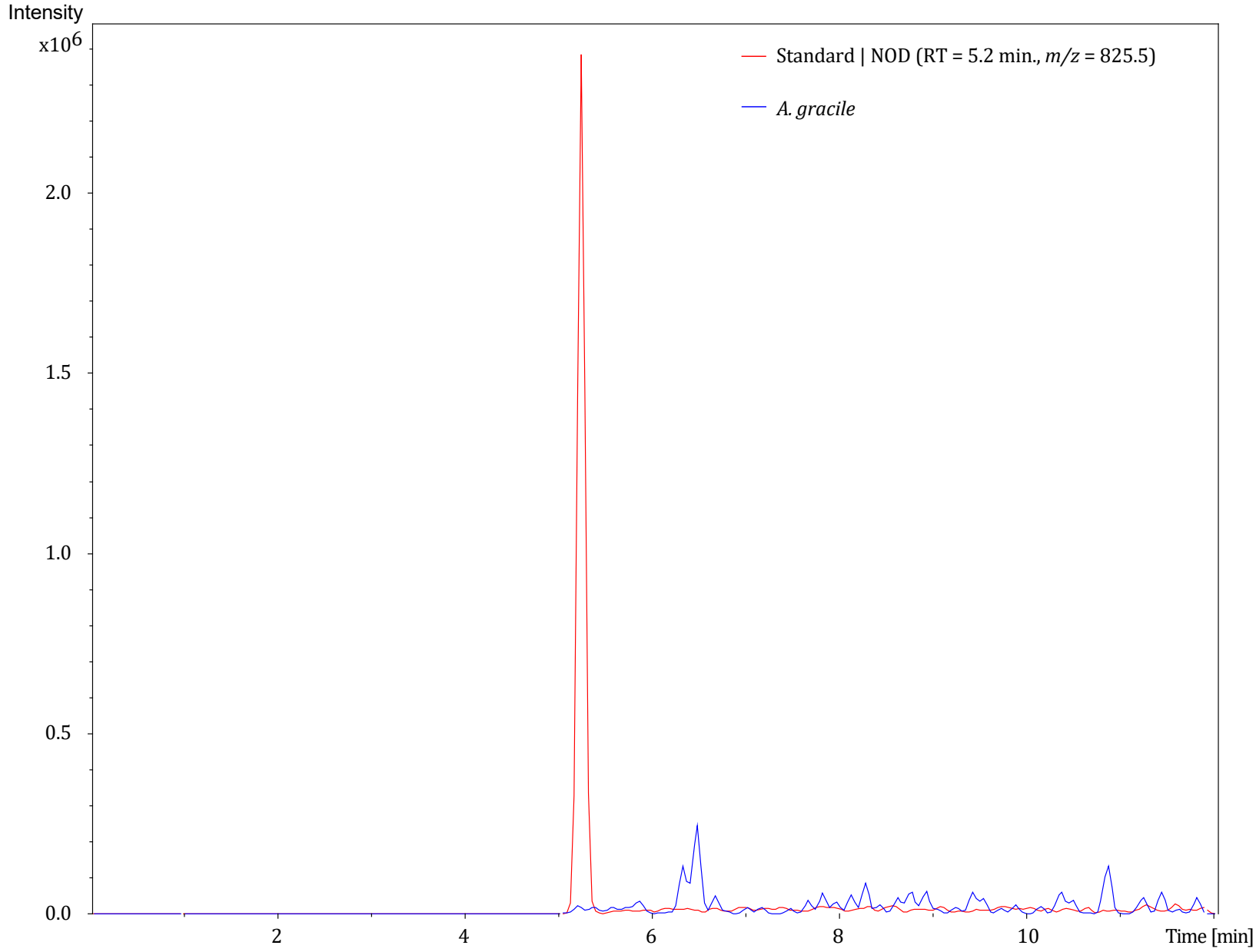

The results of LC-MS analysis | extracted ion chromatogram of NOD standard and *Planktothrix agardhii* SAG 6.89