

Identification and Isolation of DSP-Toxins Using a Combined LC/MS-System for Analytical and Semipreparative Work

Application

Food Safety

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Abstract

The configuration and operation of a combined liquid chromatography/mass spectrometry (LC/MS) system to identify and isolate DSP-toxins is described. In the analytical mode, okadaic acid (OA) and dinophysistoxin-1 (DTX-1) are more selectively and sensitively monitored when compared to LC with fluorescence detection. With less sample preparation, the detection limits are decreased by a factor of 3–5, depending on the matrix. In semipreparative mode, OA and DTX-1 could be isolated from crude extracts of *Prorocentrum lima* algae using mass-based fraction collection with a purity >98%. Due to this method, reference standards of DSP toxins are now commercially available.

Introduction

Diarrhetic shellfish poisoning (DSP) is a gastrointestinal syndrome that occurs in humans after the consumption of bivalve mollusks such as scallops,

mussels, clams and oysters. The symptoms include abdominal pain, vomiting, nausea, headache, diarrhea, chills, and fever. DSP toxins can be classified in three groups: the okadaic acid (OA) group involving OA and the dinophysistoxins (DTXs), the pectenotoxin group (PTXs) and the yessotoxin group (YTXs).

Inside the OA group, OA and DTX-1 are the main toxins responsible for DSP outbreaks. The outbreaks led to the establishment of control programs for marine biotoxins in many countries. In Germany residues of DSP toxins in mussels are controlled at present under the regulation of the Fischhygiene-Verordnung of 8th June 2000. This Order requires the testing of shellfish for the presence of toxins by means of animal tests (mouse bioassays) or by chemical analytical procedures [1, 2]. Liquid chromatography with fluorescence detection is an established technique, but it requires the derivatization of the not naturally fluorescent DSP toxins. Using LC/MS coupled with electrospray ionization (ESI) more sensitive and selective results are attainable with less sample preparation.

The greatest problem regarding the analytical methods for monitoring DSP toxins is the availability of pure reference material. The DSP toxins OA and DTX-1 can be isolated from crude extracts of *Prorocentrum lima* algae (see Figure 1) using mass-based fraction collection in semipreparative mode. The present work describes the configuration, setup, and operation of a combined LC/MS system for analytical and semipreparative work.



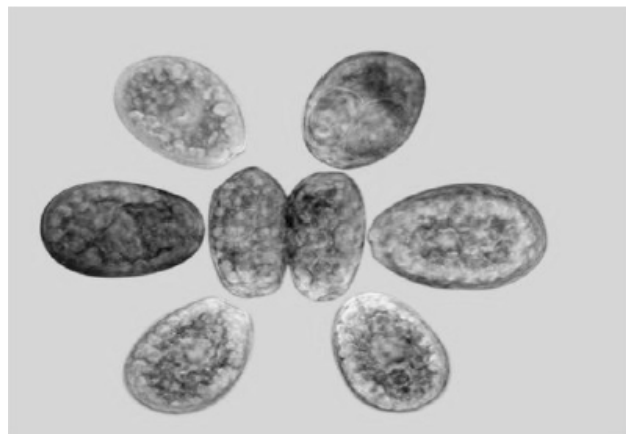


Figure 1. *Prorocentrum lima* algae under the microscope.

Experimental

The DSP toxins shown in Figure 2 were analyzed in this work. The analyses were conducted in two modes: Analytical and Semipreparative.

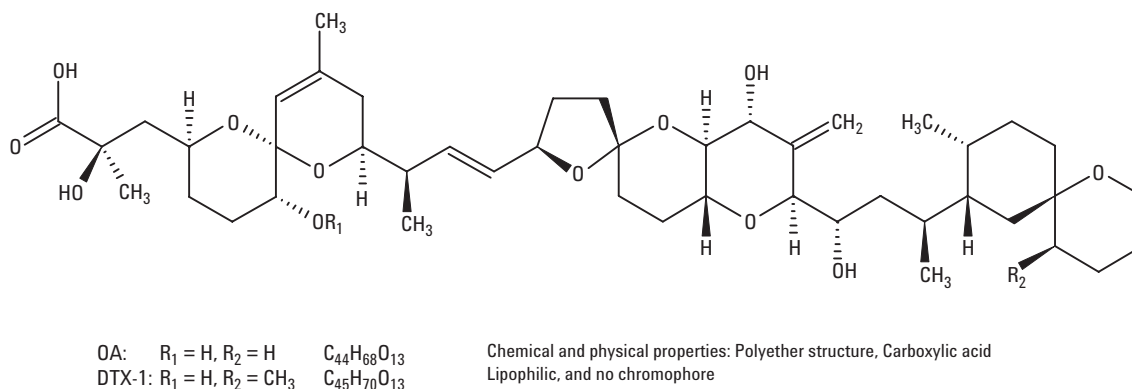


Figure 2. DSP toxins.

LC/MS Method Details - Analytical

LC Conditions

Instrument:	Agilent 1100 HPLC (Quaternary pump)
Column:	150 × 3.0 mm ZORBAX SB-C18, 5 μm
Mobile phase:	A Water (0.1% Formic acid) B Methanol
Gradient:	20% B at 0 min 20% B at 5 min 80% B at 20 min
Stop time:	28 min;
Post time:	4 min
Flow rate:	0.6 mL/min
Injection vol:	10 μL

MS Conditions

Instrument:	Agilent LC/MSD
Source:	Positive/Negative switching ESI
Drying gas flow rate	12 L/min
Nebulizer:	60 psig
Drying gas temp:	350 °C
V_{cap} :	3000 V (positive and negative)

LC/MS Method Details - Semipreparative

LC Conditions

Instrument 1:	Agilent 1100 HPLC (Quaternary pump)
Column:	50 × 9.4 mm ZORBAX SB-C18, 5 μm
Mobile phase:	A Water (0.1% Formic acid) B Methanol
Gradient:	20% B at 0 min 20% B at 5 min 80% B at 20 min
Stop time:	28 min
Post time:	4 min
Flow rate:	7.0 mL/min
Injection vol:	100 μL (250 μL using Multiple Draw Mode)
Instrument 2:	Agilent 1100 HPLC (Isocratic pump) for makeup flow
Flow rate:	0.8 mL/min (50% H ₂ O + 50% MeOH + 0.1% Formic acid)
Active splitter:	Split ratio 271:1

MS Conditions

Instrument:	Agilent LC/MSD
Source:	Negative ESI
Drying gas flow:	12 L/min
Nebulizer:	60 psig
Drying gas temp:	350 °C
V _{cap} :	3000 V (positive)
MSD Fraction Collection Setup	
FC Mode:	Use method target mass; Adducts: (M-H) ⁻

Results and Discussion

Analytical Work

In the analytical mode of the LC/MS system (Figure 3) the DSP toxins were monitored using ESI with positive/negative mode switching. The positive ion mode is four times more sensitive than the negative ion mode (Figure 4). Mass spectra for OA and DTX-1 show a sodiated molecular ion instead of a protonated molecular ion, and characteristic fragment ions $[M+H - nH_2O]^+$, where $n = 1-4$, formed by a sequential loss of water. In negative ion mode only the $[M-H]^-$ ion is detected. LC/MS provided a more selective and sensitive method for monitoring DSP toxins in comparison to LC with fluorescence detection (Figure 5), by a factor of 3–5.

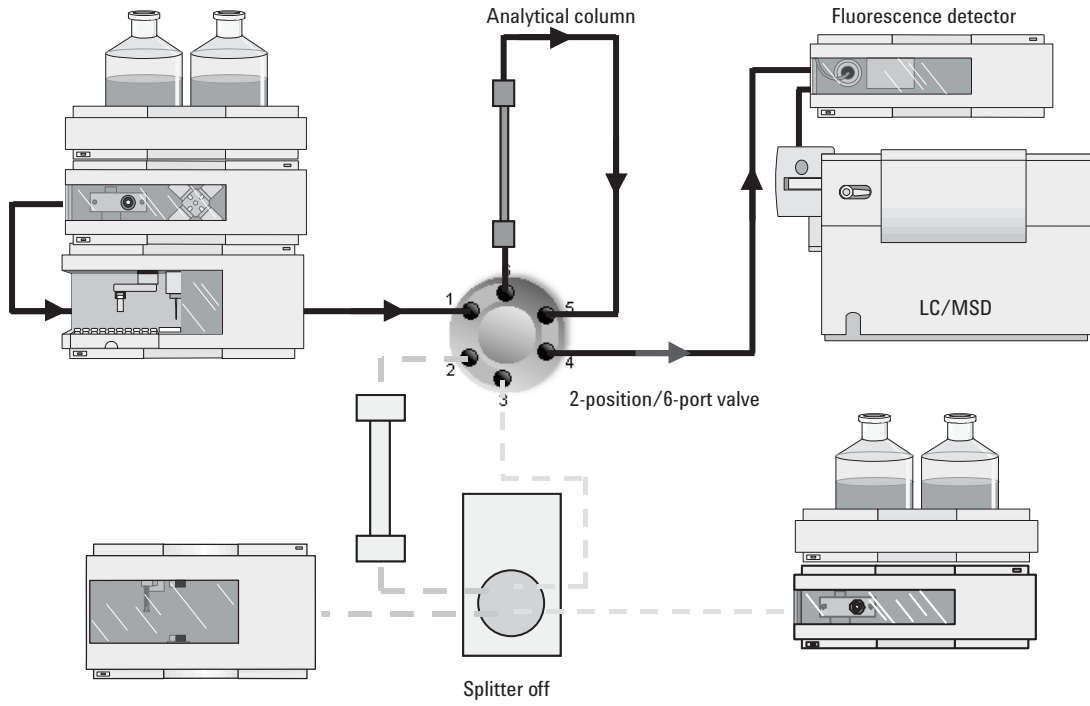


Figure 3. System diagram (analytical work).

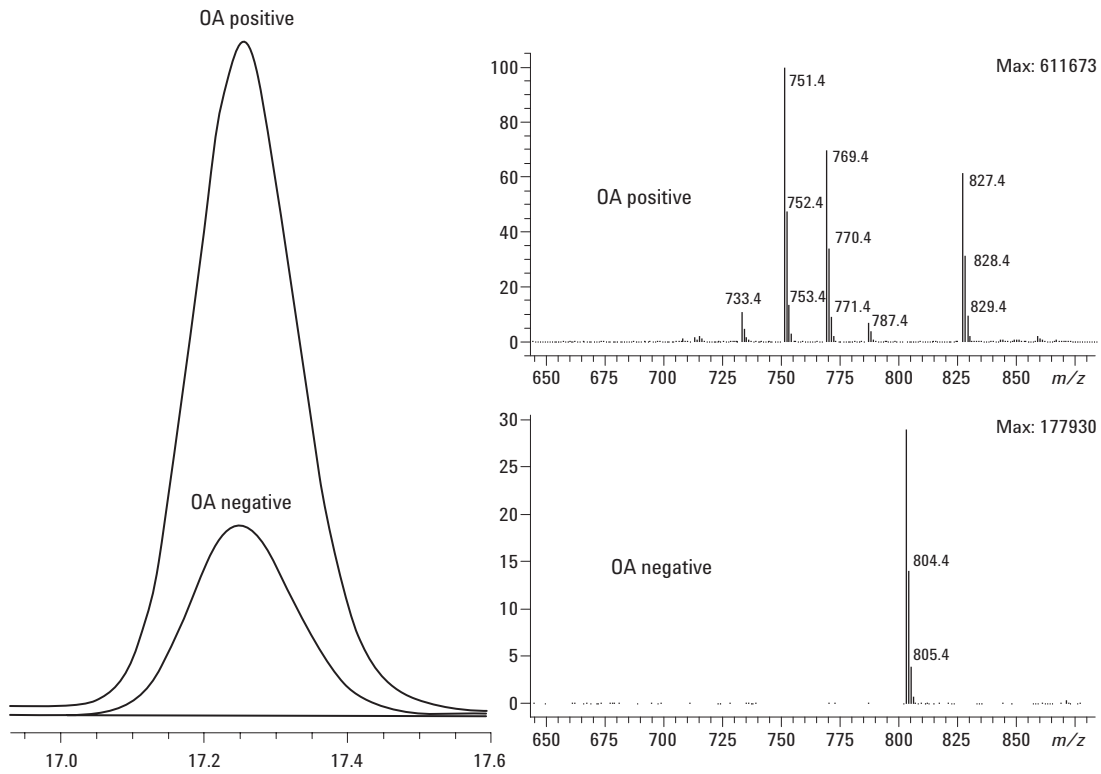


Figure 4. LC/MS analysis of OA.

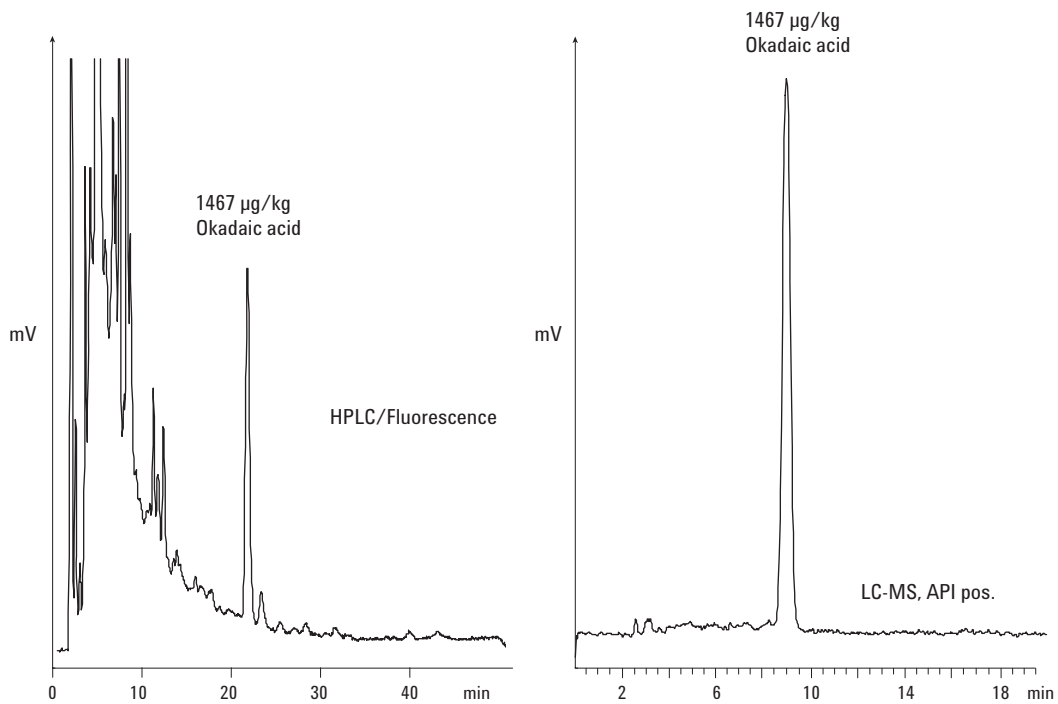


Figure 5. Comparative analysis of DSP toxins in shellfish.

Semipreparative Work

The reference standards could be obtained by switching the system to semipreparative mode (Figure 6). The valve is switched to position 2.

The main flow now goes to the semipreparative column and then through the splitter to fraction collector (AS). The make-up flow goes through the splitter where it picks up some of the compound from the main flow and goes to the MS-detector (MSD).

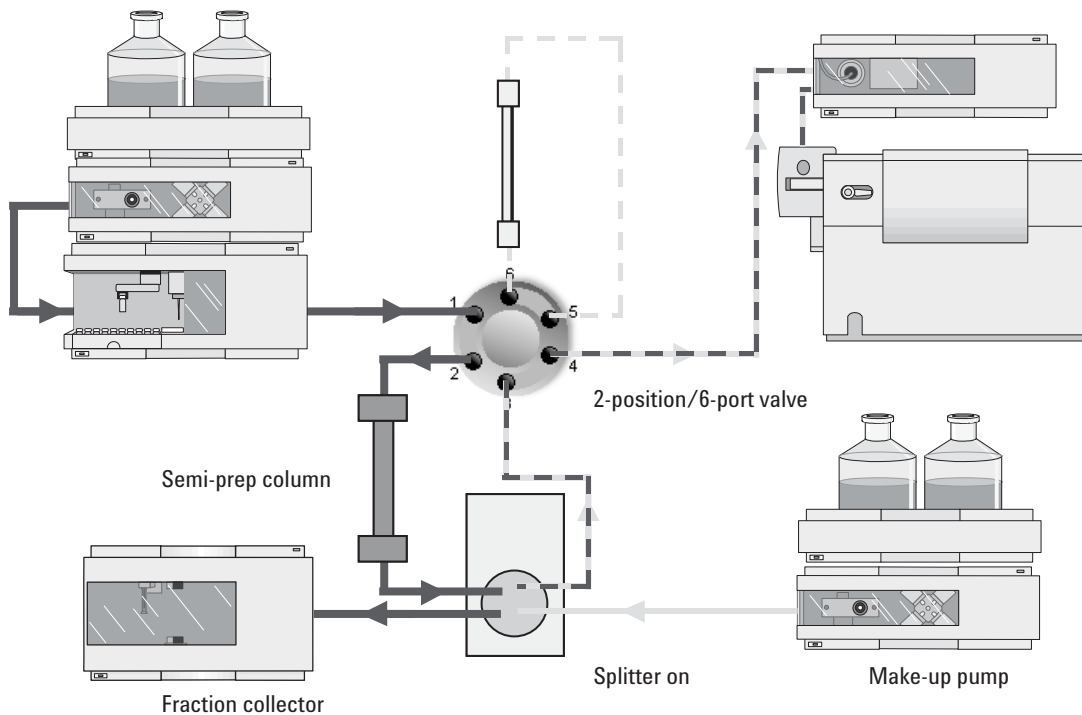


Figure 6. System diagram (semipreparative work).

Besides OA and DTX-1, a new OA-toxin with similar mass spectral properties could be isolated from crude extracts of *Prorocentrum lima* algae using mass-based fraction collection (Figure 7). The mass-based fraction collection of a methanolic extract of *Prorocentrum lima* algae results in three fractions: OA, DTX-1 and an unknown toxin. From MSⁿ experiments it can be determined that the molecular structure of the unknown toxin must be very similar to those of OA and DTX-1.

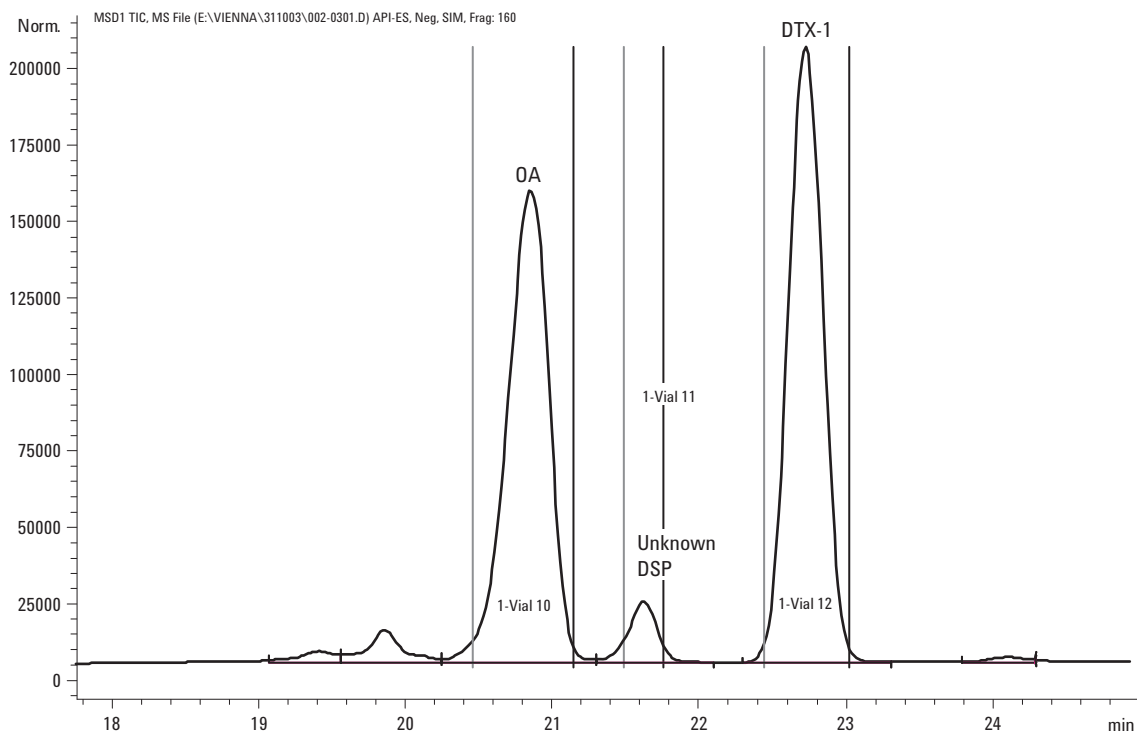


Figure 7. Mass-based fraction collection of DSP toxins.

Because of the low concentration, the target compounds had to be collected from multiple injections of the same sample, a process usually referred to as pooling (Figure 8). Reanalysis of the collected fractions gave results for purity >98%. This method is robust (Figure 9) and has now resulted in making reference standards of DSP toxins commercially available.

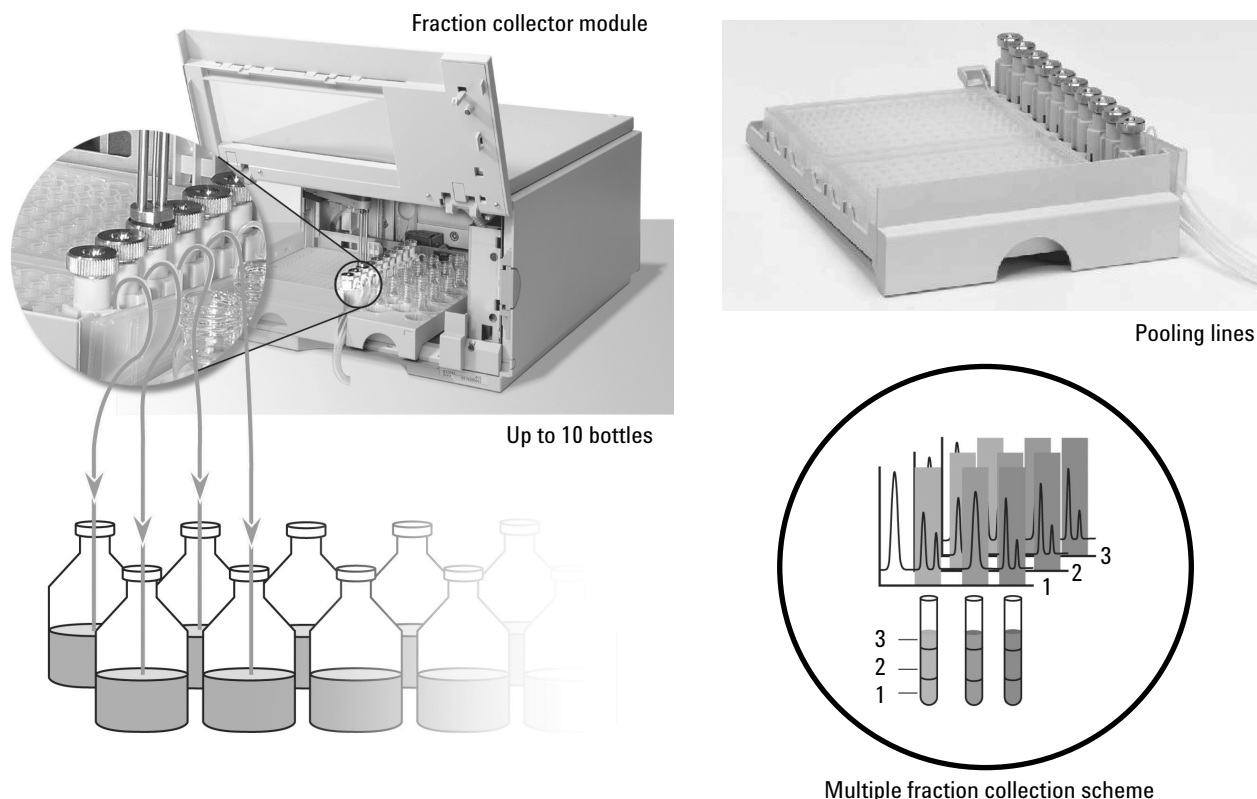


Figure 8. Pooling.

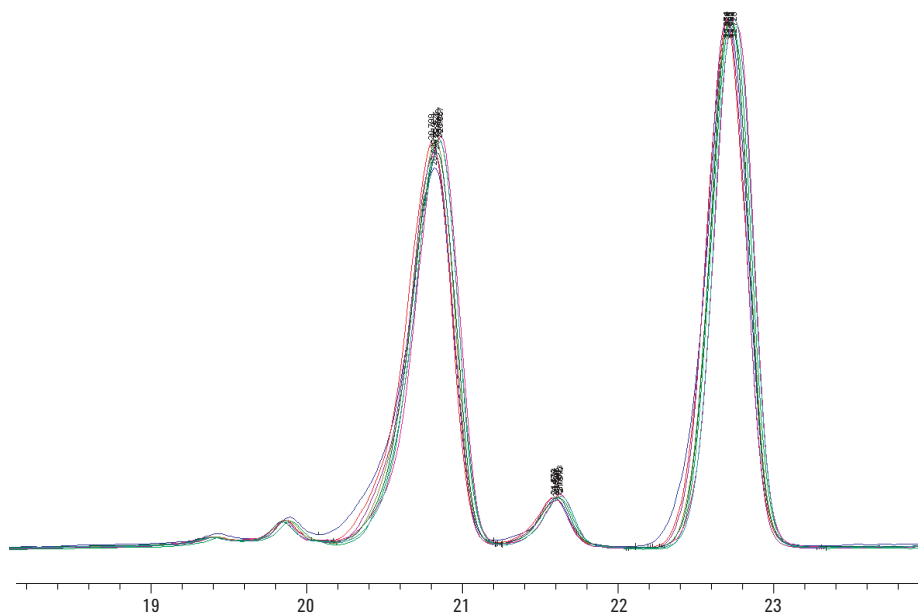


Figure 9. Robustness of the method - overlay of 10 mass-based fraction collection runs.

Conclusions

Configuration and operation of a combined LC/MS system to identify and isolate DSP toxins is described. In the analytical mode, OA and DTX-1 were monitored more selectively and sensitively than by using LC with fluorescence detection. With less sample preparation, the detection limits could be decreased by a factor of 3–5, depending on the matrix. In the semipreparative mode OA and DTX-1 could be isolated from crude extracts of *Prorocentrum lima* algae using mass-based fraction collection with a purity >98%. Due to this method reference standards of DSP toxins are now commercially available.

References

1. M.A. Quilliam, A. Gago-Martinez, and J.A. Rodriguez-Vasquez, "Improved method for preparation and use of 9-anthryldiazomethane for derivatization of hydroxycarboxylic acids - Application to diarrhetic shellfish poisoning toxins", (1988), *Journal of Chromatography A*, **807**, 229–239.
2. A.G. Bauder; A.D. Cembella, V.M. Bricelj, and M.A. Quilliam, "Uptake and fate of diarrhetic shellfish poisoning from the dinoflagellate *Prorocentrum lima* in the bay scallop *Argopecten irradians*", (2001), *Marine Ecol. Progr. Ser.*, **213**, 39–52.

For More Information

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