Determination of Paralytic Shellfish Poisoning Toxins via ZIC®-HILIC and MS/MS

Paralytic Shellfish Poisoning toxins are very polar compounds that can be separated via a ZIC®-HILIC stationary phase. A single run is capable of monitoring eighteen toxins with superb limits of detection (typically <0.5 ng, S/N = 3) and good linearity using a triple quadrupole mass spectrometer.

Introduction

Paralytic shellfish poisoning (PSP) toxins, which are synthesized by marine dinoflagellates and cyanobacteria accumulate in shellfish such as mussels. PSP toxins are powerful sodium channel blockers that can cause respiratory insufficiency and death. An AOAC mouse bioassay used to determine PSP's only determines the total toxicity of the sample. Chromatographic separation is used to identify individual toxins. Some methods are tedious (e.g. involve pre-chromatographic oxidation, post column derivatization or the need for multiple separations) while others do not separate all of the toxins, or utilizes ion-pair reagents.

In this technical note, a new analytical method for the determination of paralytic shellfish toxins using hydrophilic interaction liquid chromatography is described. This method provides a sensitive and selective tool which can be employed with either a fluorescence detector or a mass spectrometric detector. The method can be used for various phytoplankton and the routine analysis of seafood.

Hydrophilic interaction liquid chromatography (HILIC) is a very powerful technique for the separation of complex mixtures of polar compounds. It uses a hydrophobic mobile phase with a hydrophilic stationary phase. The solutes elute in order of hydrophilicity, the opposite of the elution order in RP and the stationary phase is especially useful for the separation of polar compounds that are poorly retained by RP.

ZIC®-HILIC chromatography is a unique form of HILIC where a bonded zwitterionic sulfobetaine group acts as the interactive layer. The low reactivity and zwitterionic properties of the group makes the ZIC®-HILIC a logical choice for the separation of the extremely polar toxins.

Experimental

Standards, Reagents and Eluants: PSP standards were obtained from National Research Council of Canada, Marine Analytical Chemistry Standards Program. Reagents and eluants were obtained from commercial sources and used as received. Water was obtained from a Millipore system.

Extraction of Toxins: Shellfish samples were homogenized with 0.2 M HCl with an ultrasonic probe and heated (90°C) for 15 min to convert N-sulfocarboamoyl toxins to the corresponding carbamoyl toxins). The suspension was centrifuged for 10 min and the supernatant was filtered. PSP toxins were extracted from marine diflagellates using 0.03 M HOAc.

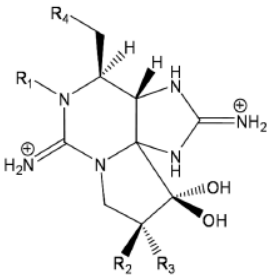

Figure 1: General Structure of Paralytic Shellfish poisoning toxins. R1 = H or OH, R2 = H or OSO_3^-, R3 = H or OSO_3^- see Table 1 for identification

Separation/Detection: Samples were separated via a HPLC system using a ZIC®-HILIC column (250 x 4.6 mm, 5 µm particles) and a formic acid gradient. Detection was via a PE Sciex Triple quadrupole mass spectrometer. For fluorescence detection (Ex=350 nm, Em=395 nm), a formate buffer/acetonitrile gradient was applied. After separation, 0.75 M nitric acid was used to lower the pH and periodic acid/ammonia was used to oxidize the PSP's.

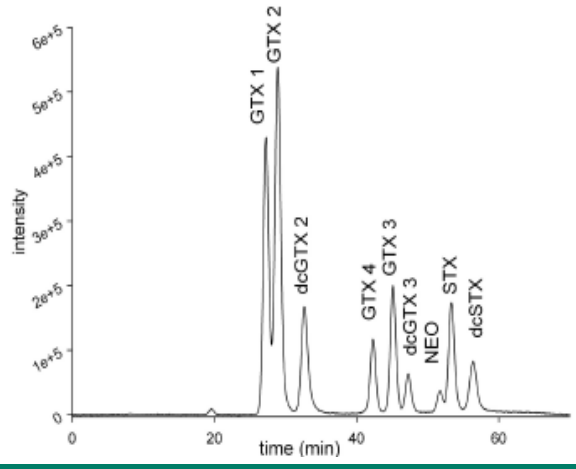

Figure 2: ZIC®-HILIC separation of PSP toxins in standard mixture with Fluorescence Detection. See text and Table 1 for more details.

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Toxin	R1	R2	R3	R4	Toxicity factor
STX	H	H	H		1
NEO	OH	H	H		0,92
GTX I	OH	H	OSO_3^-	H_2N-COO	0,99
GTX II	H	H	OSO_3^-	(Carbamoyl -)	0,36
GTX III	H	OSO_3^-	H		0,64
GTX IV	OH	OSO_3^-	H		0,73
B1	H	H	H		0,06
B2	OH	H	H		no data
C3	OH	H	OSO_3^-	^-O_3S-NH-COO	0,01
C1	H	H	OSO_3^-	(N-Sulfo -	< 0,01
C2	H	OSO_3^-	H	carbamoyl -)	0,10
C4	OH	OSO_3^-	H		0,06
dc-STX	H	H	H		0,51
dc-NEO	OH	H	H		no data
dc-GTX I	OH	H	OSO_3^-	HO	no data
dc-GTX II	H	H	OSO_3^-	(Decarbamoyl -)	0,65
dc-GTX III	H	OSO_3^-	H		0,75
dc-GTX IV	OH	OSO_3^-	H		no data

Table 1: Structure of PSP toxins.
(GTX=Gonyautoxin, NEO=Neosaxitoxin, STX=Saxitoxin)

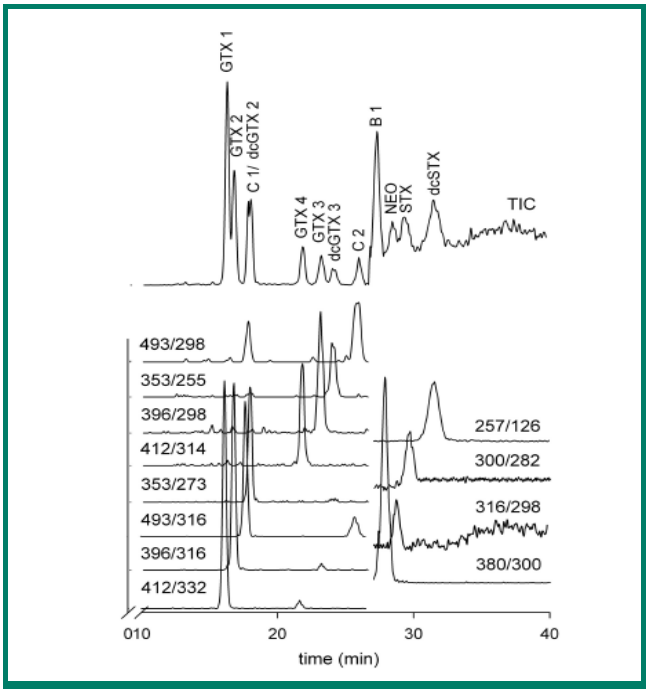

Figure 3: Separation of the standard mixture with MS/MS detection. The standard mixture contained GTX1, 6.60 ng; GTX2, 3.45 ng; dcGTX2, 3.00 ng; C1, 4.20 ng; GTX4, 2.10 ng; GTX3, 1.16 ng; dcGTX3,0.84 ng; NEO, 7.65 ng, C2, 6.00 ng; STX, 4.20 ng; B1, 4.90 ng and dcSTX, 4.80 ng

Reference

Note: This application note is condensed from the scientific paper "*Application of a new zwitterionic hydrophilic interaction chromatography column for determination of paralytic shellfish toxins"* by Diener, M; Erler, K, Christian, B and Luckas, B. J. Sep Sci. 2007, **30, 1821-1826.** (Figure 2-4 and Table 1 are reprinted from reference 1 with permission from Wiley-VCH Verlag GmbH&Co., KgaA, Weinheim, Germany).

Results

Typical chromatograms are shown in Figure 2 and 3, while Figure 4 presents the separation of the PSP toxins in a spiked *Gymodinium catenum* extract. This chromatogram clearly indicates that the method is capable of separating the analytes in an analytically useful fashion. In a similar fashion, a successful separation with fluorescence detection was obtained with spiked extract of *Alexandrium catenella.* The more selective detection with MS allowed for modification of the gradient to provide a more rapid separation. The LOD's via MS ranged from 0.04 ng (dcGTX2) to 0.60 ng (NEO), similar LOD's were obtained with fluorescence detection.

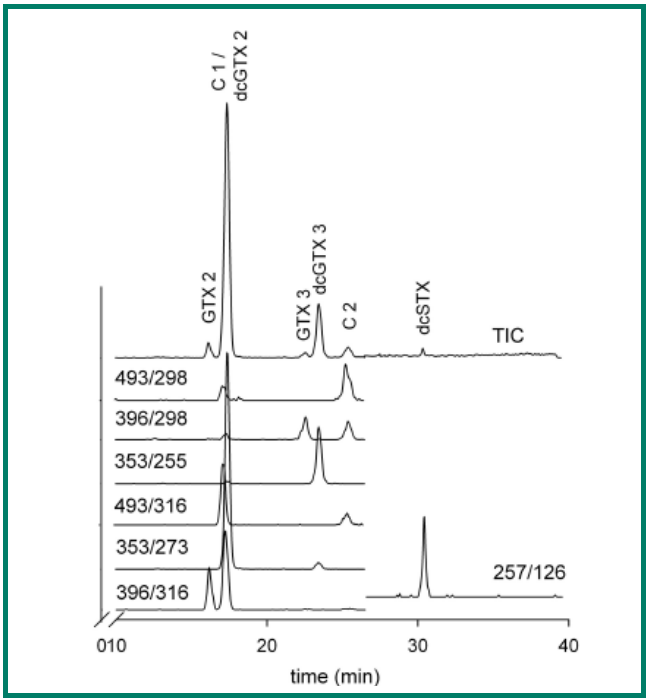

Figure 4: HILICMS/MS analysis of a Gymnodinium catenatum extract, Baja California, Mexico.

Conclusions

ZIC®-HILIC detection provides excellent resolution and sensitivity for monitoring extremely polar PSP's in biological material such as alga and mussels. A straight-forward extraction procedure and a single separation allows for the determination of a broad range of compounds with excellent sensitivity over a broad concentration range. Sub-ng LOD's can be observed with either fluorescence detection (after oxidation and the assay provides information about individual PSP toxins while the mouse bioassay provides total toxicity. The assay does not use ion-pairing reagents, so MS detection is quite straight forward.

About ZIC®-HILIC Chromatography

ZIC®-HILIC stationary phases are based on the covalently bonded permanent zwitterionic sulfobetaine group. It is available with a silica support in 3.5, 5 and 10 µm particle sizes in various column dimensions from capillary to semi-preparative (75 µm up to 20 mm ID). In addition, it is available with a polymeric support in 5 µm particles (ZIC®-*p*HILIC). MerckSeQuant also publishes the tutorial booklet A Practical Guide to HILIC, which is available free, both on-line and as a hardcopy.

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