salome.poyer@etu.univ-rouen.fr

Identification of paralytic shellfish poisons using liquid chromatography / ion mobility - high resolution mass spectrometry

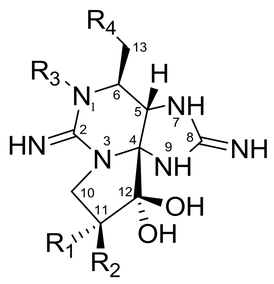
Salomé Poyer[a], Corinne Loutelier-Bourhis[a], Florence Mondeguer[b], Gaël Coadou[a], Marie Hubert-Roux[a], Julien Enche[c], Anne Bossée[c], Philipp Hess[b], Carlos Afonso[a*]

[a] *Normandie Université, COBRA, UMR 6014 et FR 3038 ; Université de Rouen ; INSA de Rouen ; CNRS, IRCOF, 1 rue Tesnière, 76821 Mont Saint Aignan Cedex, France.*
[b] *IFREMER, Laboratoire Phycotoxines, F-44311 Nantes 03, France.*
[c] *DGA Maîtrise NRBC, département Analyse Chimique, F-91710 Vert Le Petit, France.*

Introduction

Saxitoxin and its analogues also called paralytic shellfish poisons (PSPs) are very potent neurotoxins [1] produced by dinoflagellates and referenced as chemical weapon in the chemical warfare convention (CWC). The official detection methods for these toxins present limitations concerning their speed and reliability [2].

Due to the presence of isomers, not differentiable by mass spectrometry (MS), an upstream separation is necessary. In order to separate saxitoxin analogues, hydrophilic interaction liquid chromatography (HILIC)[3] and ion mobility (IM) were used. Those techniques, respectively developed for high polar compounds and three dimensional structure differentiation, are particularly well adapted to the separation of PSPs. This HILIC/IM-MS coupling was used to develop a fast, reproducible and sensitive method for the separation and the detection of the PSPs.

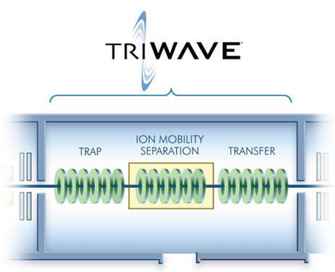


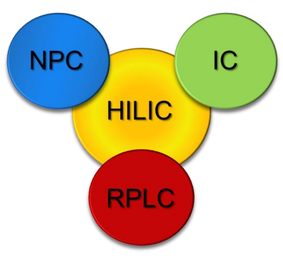
R1	R2	R3	Carbamate	Decarbamoyl	N-sulfocarbamoyl
H	H	H	**STX**	**dcSTX**	**GTX5**
H	H	OH	**NEO**	**dcNEO**	GTX6
OSO_3^-	H	H	**GTX2**	**dcGTX2**	C1
H	OSO_3^-	H	**GTX3**	**dcGTX3**	C2
OSO_3^-	H	OH	**GTX1**	dcGTX1	C3
H	OSO_3^-	OH	**GTX4**	dcGTX4	C4
	R4		H_2N-COO-	HO-	^-O_3S-NH-COO-

Analytical strategies

Ion mobility

A hybrid Q-IM-TOF instrument (Synapt G2 HDMS) was used to perform the IM-MS experiments. The travelling wave IM cell permits the separation of ions according to their gas phase size and shape. The time spent by ions in the IM cell in a buffer gas under the constraint of an electric field is measured to obtain a drift time.





HILIC

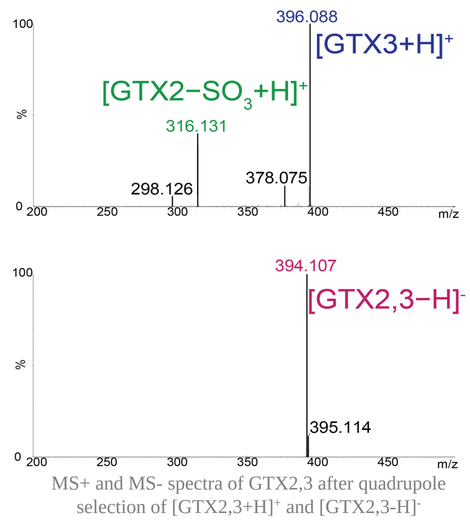
HILIC uses polar and/or charged stationary phases such as normal phase (NP) and ionic chromatography (IC) to separate polar compounds. HILIC solvents are similar to those used in reversed phase (RP) chromatography, particularly well adapted to the MS coupling. TSK gel amide 80 was used to separate the saxitoxins analogues [4].

MS results

PSPs analogues present two different features in MS detection:

- STX, NEO and their decarbamoyl derivatives are observed on their protonated forms,
- Gonyautoxins (GTXs) and decarbamoyl gonyautoxins (dcGTXs), which present sulphate group, are both detected for their $[M+H]^+$ and $[M-H]^-$ forms.

Extensive fragmentation of $[M+H]^+$ ions occurs for GTX1,2,5 and dcGTX2 yielding the $[M-SO_3+H]^+$ ions, regio-isomers of NEO, dcNEO and STX.
No fragmentation of $[M-H]^-$ were observed.

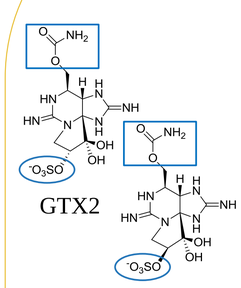


MS+ and MS- spectra of GTX2,3 after quadrupole selection of $[GTX2,3+H]^+$ and $[GTX2,3-H]^-$

Results

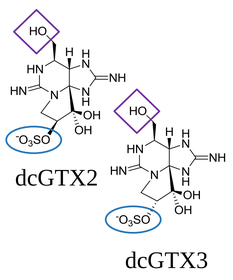
HILIC-MS

Optimization of the LC parameters was carried out using two different HILIC phases; silanol with the Alltima (2.1 × 150 mm × 3µm) and amide with the TSK gel amide 80 (2 × 250 mm × 5µm). Best results for the separation of diastereomers were obtained with the amide 80 column.
In optimum conditions, STX, NEO and their decarbamoyl derivatives are not separated and present high peak width. Due to the $[GTX+H]^+$ fragmentation, GTXs and dcGTXs were detected on negative ion mode, whereas non-sulphated species were detected in positive ion mode detection.



IM-MS

Ion mobility parameters were optimized to obtain the best separation over resolution ratio for the PSPs. These results were obtained by injection of 1 µg.mL^{-1} toxins mixture and 1 µg.L^{-1} of each toxin in order to circumvent the fragmentation involving regio-isomers.
STX, NEO, dcSTX, dcNEO and GTX5 were differentiated. The three isomeric pairs were also differentiated but no diastereomers separation was observed for both ionization modes without further separation.

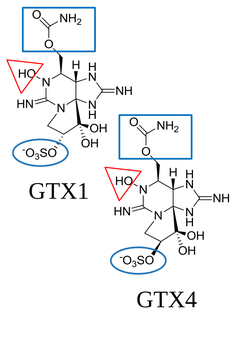


HILIC/IMS-MS

This three dimensional separation was considered due to off-line epimerization and complementarity observed on the previous results:

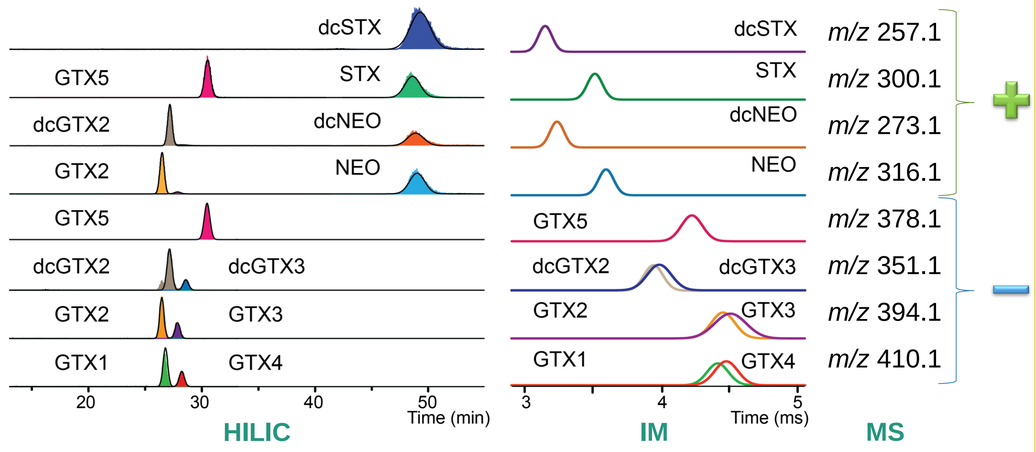
- Diastereomers separation with HILIC/MS
- Non-sulphated analogues separation using IM-MS coupling.

The HILIC/IM-MS coupling was studied for both ionization modes. Analogues including isomers were differentiated for the two ionization modes. Experimental drift times order were inversed depending of the ionization mode, which indicate different conformation based on the GTXs charge state :



$[GTX1+H]^+ > [GTX2+H]^+ > [GTX3+H]^+ = [GTX4+H]^+$
$[GTX1-H]^- < [GTX2-H]^- < [GTX3-H]^- = [GTX4-H]^-$

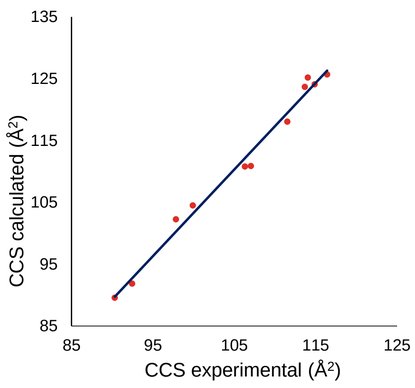
HILIC/IM-MS spectra of PSPs mixture



HILIC conditions: TSK gel amide 80 (1 × 250 mm × 5 µm) Solvent A : 2 mM ammonium acetate pH 3.5, Solvent B : MeCN Injection 1 µL, Temperature 40 °C, Flow rate 30 µL/min. Gradient : 0 min (80% B), 40 min (30% B), 45 min (30% B), 46 min (80% B), 60 min (80% B).

IM conditions: Helium cell gas flow 180 mL.min^{-1}, N_2 gas flow 90 mL.min^{-1}, wave height 40 V, wave velocity 900 m.s^{-1}
MS conditions +/-: Capillary voltage 3.5/2.1 kV, sampling cone voltage 45/50 V, extraction cone 4.7/4 V, desolvation gas flow 650/850 L.Hr^{-1}

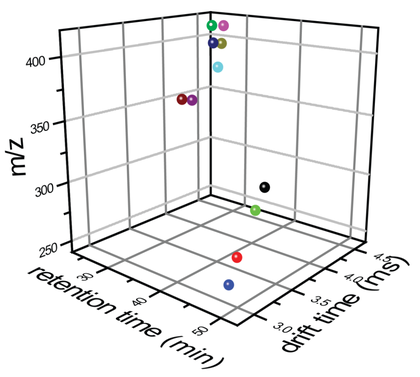
Collision cross sections (CCS) determination



Dynamic modelling was performed using simulating annealing technic on 50 different neutral structures. CCS were estimated using trajectory method algorithm of the Mobcal software [5].
Experimental drift times were converted on CCS values by calibrating the TWIM cell with polyalanine. A good correlation was observed (R^2 = 0.9872) between CCScalc and CCSexp with a systematic error giving a slope of 1.4.

Conclusion and perspectives

- On-line HILIC/IM-MS coupling afforded a three dimensional separation of the PSPs analogues without any epimerisation. This technique is fast and reproducible on standard molecules. This coupling has to be adapted to complex matrices such as mussels and oyster. It was demonstrated that the injection of complex samples by HPLC/MS results in important matrix effects and a loss of reproducibility. The use of IM-MS is known to be more reproducible than HPLC/MS when complex mixtures are used due to the separation process which is non-environmental dependant.
- The use of different columns dimensions such as ultra high performance liquid chromatography columns will be tested in order to decrease the duration of analysis. Optimization on different HILIC columns and off/on line SPE purification will be explored to overcome matrix effects.
- Experimental CCS values were determined for STX and analogues. CCS calculation on neutral species presented a good correlation with experimental values. Theoretical CCS will be determined on charged species in order to understand the PSPs behaviour depending on their charge state.



References

[1] Wiese, M. *et al.*, *Marine Drugs* **2010,** *8* (7), 2185-2211., **[2]** a- Lawrence, J. F. *et al.*, *J AOAC Int* **1995,** *78* (2), 514-20., b- Oshima, Y., *J.AOAC Int.* **1995,** *78*, 528-532., c- *Official Methods of Analysis* (1990) 15th Ed., AOAC, Arlington, VA, sec. **959.08**., **[3]** Halme M. et al., *J. Chromatogr. B* **2012,** 880, 50-57., **[4]** Dell'Aversano C. *et al.*, *J. Chromatogr.* ***2005,*** 1081, 190-201., **[5]** Mesleh, M. F. *et al.*, *J. Phys. Chem.* **1996**, *100*, 16082.

Acknowledgements

The authors acknowledge the région Haute-Normandie, European Regional Development Fund (ERDF 31708) and direction générale de l'armement (DGA) for financial supports.