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

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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 saxitoxins, 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 developed to develop a fast, reproducible and sensitive method for the separation and the detection of the PSPs.

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 not separated and present high peak width. Due to the [GTX-H]+ fragmentation, GTX2 and GTX3 were detected on negative ion mode, whereas non-sulfated species were detected in positive ion mode.

- Extensive fragmentation of [M+H]+ ions occurs for GTX1,2,5 and GTX2 yielding the [M-SO3-H]+ ions, regio-isomers of NEO, deNEO and STX. No fragmentation of [M-H] were observed.

Results

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 derivates are not separated and present high peak width. Due to the [GTX-H]+ fragmentation, GTX2 and GTX3 were detected on negative ion mode, whereas non-sulfated species were detected in positive ion mode.

HILIC/IM-MS coupling

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

- Diastereomers separation with HILIC/IM-MS
- Non-sulfated 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 was inverted depending of the ionization mode, which indicate different conformation based on the GTXs charge state:

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\text{[GTX1+H]+} \rightarrow \text{[GTX2+H]+} \rightarrow \text{[GTX3+H]+} = \text{[GTX4+H]+}
\]

\[
\text{[GTX1-H]-} \rightarrow \text{[GTX2-H]-} \rightarrow \text{[GTX3-H]-} = \text{[GTX4-H]-}
\]

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-line SPE purification will be explored to overcome matrix effects.

- Experimental CS results 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


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