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High resolution mass spectrometry for quantitative analysis and untargeted screening of algal toxins in mussels and passive samplers

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ABSTRACT

Measurement of marine algal toxins has traditionally focussed on shellfish monitoring while, over the last decade, passive sampling has been introduced as a complementary tool for exploratory studies. Since 2011, liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been adopted as the EU reference method (No. 15/2011) for detection and quantitation of lipophilic toxins. Traditional LC–MS approaches have been based on low-resolution mass spectrometry (LRMS), however, advances in instrument platforms have led to a heightened interest in the use of high-resolution mass spectrometry (HRMS) for toxin detection. This work describes the use of HRMS in combination with passive sampling as a progressive approach to marine algal toxin surveys. Experiments focused on comparison of LRMS and HRMS for determination of a broad range of toxins in shellfish and passive samplers.

Matrix effects are an important issue to address in LC–MS; therefore, this phenomenon was evaluated for mussels (*Mytilus galloprovincialis*) and passive samplers using LRMS (triple quadrupole) and HRMS (quadrupole time-of-flight and Orbitrap) instruments. Matrix-matched calibration solutions containing okadaic acid and dinophysistoxins, pectenotoxin, azaspiracids, yessotoxins, domoic acid, pinnatoxins, gymnodimine A and 13-desmethyl spirolide C were prepared. Similar matrix effects were observed on all instruments types. Most notably, there was ion enhancement for pectenotoxins, okadaic acid/dinophysistoxins on one hand, and ion suppression for yessotoxins on the other. Interestingly, the ion selected for quantitation of PTX2 also influenced the magnitude of matrix effects, with the sodium adduct typically exhibiting less susceptibility to matrix effects than the ammonium adduct. As expected, mussel as a biological matrix, quantitatively produced significantly more matrix effects than passive sampler extracts, irrespective of toxin. Sample dilution was demonstrated as an effective measure to reduce matrix effects for all compounds, and was found to be particularly useful for the non-targeted approach.

Limits of detection and method accuracy were comparable between the systems tested, demonstrating the applicability of HRMS as an effective tool for screening and quantitative analysis. HRMS offers the advantage of untargeted analysis, meaning that datasets can be retrospectively analyzed. HRMS (full scan) chromatograms of passive samplers yielded significantly less complex data sets than mussels, and were thus more easily screened for unknowns. Consequently, we recommend the use of HRMS in combination with passive sampling for studies investigating emerging or hitherto uncharacterized toxins.

1. Introduction

A number of micro-algae produce marine toxins that can be accumulated in filter-feeding shellfish species such as mussels and oysters, and thus lead to human intoxication through consumption [1]. For several decades, the complexity of the toxins produced by these algae has impeded method development due to the lack of reference calibrants and materials. Therefore, generic mouse bioassays were often used, despite commonly accepted drawbacks [2]. Liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) has become a versatile tool for the analysis of food and environmental contaminants, including toxins. LC–MS/MS is now the reference method for the detection and quantitation of

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toxins produced by harmful algae [3]. To achieve this goal, different studies have developed and validated quantitative methods for the analysis of phycotoxins, typically using low resolution mass spectrometry (LRMS) [4–9]. This technique is now being increasingly used for monitoring [10,11] and for characterization of reference materials [12,13]. Additionally, methods using high resolution mass spectrometry (HRMS) have recently been developed and quantitatively validated for some marine toxins [14–16].

However, an important issue to address when developing or validating a quantitative analytical method using LC–MS *via* electrospray (ESI) and atmospheric pressure ionization (API) sources is the possible occurrence of matrix effects [17,18]. Matrix effects are considered to be an alteration in analyte response due to the presence of co-eluting compounds, either due to mass interference (isobaric compounds) or alteration of the desorption/ionization efficiency due to co-elution. These co-eluting compounds may increase (ion enhancement) or reduce (ion suppression) the desorption/ionization of the targeted analyte [19,20]. Matrix effects may arise from different co-eluting components: endogenous compounds already present as sample constituents and still present after extraction or sample pre-treatment, or from reagents added to the mobile phase to improve chromatographic separation and peak shape [21], as well as from interfering materials used during extraction procedures or even from variable elution flow-rates [22]. Matrix effects can be easily detected when comparing the response obtained from standard solutions to those from spiked matrix extracts. In the presence of matrix effects, both identification and determination of analytes can be affected [22]. Therefore, the evaluation of matrix effects in MS detection and solutions to overcome them should be examined in the early stages of development of new methods. Several approaches have been used to alleviate matrix effects in the quantitative analysis of lipophilic marine toxins. These approaches include SPE cleanup and column flushing [23,24], matrix-matched calibration and standard addition [24–26], reduction of the injection volume [11], use of an internal standard and use of a different ionization source such as APCI [19].

For applications that require analyses of complex biological samples, the use of HRMS can offer at least two major advantages: (i) the ability to overcome mass interferences stemming from overlapping signals of isobaric species (at low resolution such interferences lead to overestimation of the quantity of the analyte present) and (ii) non-targeted screening (where mass spectrometry is used to survey the contents of a complex mixture). In the field of toxins a good example of HRMS dealing with interfering isobaric compounds is the case of anatoxin-a, which may be hampered by the presence of phenylalanine [27]. HRMS has also been the prime technique for non-targeted screening of complex samples for unknowns, employing Orbitrap and Time-of-Flight mass spectrometers [9,28,29].

While monitoring of biotoxins has traditionally been carried out in mussels, passive samplers, also referred to as solid phase adsorption toxin tracking (SPATT) have been more recently introduced to detect toxins in the marine environment [30]. Subsequently, many studies have successfully implemented passive sampling, using mainly the HP20 resin, to detect lipophilic toxins in different aquatic environments [31–35]. This technique has not yet proven to be useful as a monitoring tool for early warning of harmful algal blooms [36]. However, passive samplers have the advantage that unlike in mussels, the adsorbed toxins do not undergo biotransformation. Mussels have traditionally been used in many monitoring programs since they can be classified as a sentinel species due to the relatively unselective feeding of mussels compared to other bivalve mollusks, e.g. oysters.

In this study, we evaluate and compare matrix effects caused by mussel matrix and passive sampler components in the analysis of different phycotoxins, using both low and high resolution mass spectrometers. As a complement to the overall non-targeted approach employing HRMS, a range of toxins was investigated quantitatively: from relatively hydrophilic toxins such as domoic acid (DA) and yessotoxins (YTX and homo-YTX), over toxins of intermediate lipophilicity such as pinnatoxins E, F and G (PnTX-E, -F, -G), gymnodimine A (GYM-A), 13-desmethylspirolide-C (13-desmeSPX-C), to the more lipophilic ones including azaspiracids 1 to 3 (AZA1, -2, -3), okadaic acid (OA) dinophysistoxins 1 and 2 (DTX1, -2), pectenotoxin 2 (PTX2) and brevetoxin-1 and 2 (BTX1, -2). A chromatographic separation method was developed and optimized to obtain good separation of the toxins of interest. Matrix matched calibration curves, prepared using mussel and passive sampler extracts, were injected on different analytical systems with low resolution (triple quadrupole) and high resolution (Orbitrap and quadrupole time-of-flight) mass spectrometers. The impact of the ion selected for quantitation, sample dilution and use of low or high resolution detectors on matrix effects were assessed. Finally, the study evaluated the benefits of passive sampler matrix as a complementary tool to traditionally used shellfish matrix (mussels) with the help of HRMS for an untargeted, exploratory approach.

2. Experimental

2.1. Chemicals and reagents

Certified calibration solutions were from the National Research Council of Canada (NRCC, Halifax, NS, Canada). These included calibration solution CRMs: domoic acid (DA), azaspiracids 1, 2 and 3 (AZA1–3), pectenotoxin 2 (PTX2), okadaic acid (OA) dinophysistoxins 1 and 2 (DTX1 and -2), yessotoxin (YTX), homo-yessotoxin (homo-YTX), 13-desmethyl spirolide C (13-desmeSPX-C), pinnatoxin G (PnTX-G) and gymnodimine A (GYM-A); and mussel tissue CRMs: CRM-ASP-Mus-d, CRM-DSP-Mus-c and CRM-AZA-Mus. A multitoxin tissue material CRM-FDMT-1 undergoing certification, well-characterized in-house calibration solutions for PnTX-E and F, brevetoxins 1 and 2 (BTX1 and -2), 20-methyl spirolide G (20-me-SPX-G) and pectenotoxin-2-seco acid (PTX2sa), as well as a mussel extract (Bruckless, Donegal, Ireland – 2005) containing different azaspiracids were also provided by NRC.

Alexandrium ostenfeldii (*A. ostenfeldii*) extract containing 13,19-didesmethyl spirolide C (13,19-didesme-SPX-C) and *Ostreopsis ovata* (*O. ovata*) extract containing ovatoxin-a (OvTX-a) were obtained from Ifremer as previously described [33,37]. Those extracts were mixed with some of the abovementioned certified and in-house reference toxin calibration solutions as well as the mussel extract from Bruckless to obtain a composite multi-toxin sample, used for optimization of chromatographic separation.

HPLC-grade methanol, acetonitrile and formic acid (98%) were obtained from Sigma–Aldrich (Steinheim, Germany) and Caledon (Georgetown, ON, Canada). Ammonium formate was from Fluka (St. Louis, MI, USA). Milli-Q water was produced in-house at 18 MΩ/cm quality, using a Milli-Q integral 3 system (Millipore). For analyses with HRMS instruments, acetonitrile and water of LCMS-grade were obtained from Fisher Scientific (Illkirch, France).

2.2. Instrumentation and analytical methods

2.2.1. LC–MS/MS systems

2.2.1.1. System A: triple quadrupole (QqQ). An Agilent HPLC 1100 series system (1.58 min dwell time) was connected to an API4000™ mass spectrometer (AB Sciex) equipped with a TurboIonSpray™ ionization source. For quantitation, the mass spectrometer was operated in MRM mode, scanning two transitions for each toxin. Q1 and Q3 resolutions of the instrument were set at Unit (arbitrary terms). Data were acquired in scheduled MRM and the target