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Short communication

Pre- versus post-column oxidation liquid chromatography fluorescence detection of paralytic shellfish toxins

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ABSTRACT

Both pre- and post-column oxidation liquid chromatography methods with fluorescence detection are available for detecting paralytic shellfish toxins. Each method has been evaluated in multiple laboratories and validated as a potential alternative to the mouse bioassay. This communication compares the advantages and limitations of both methods. For a given laboratory, the selection of either method may be based primarily on practicality and less on any deficiencies in scientific merit.

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1. Short communication

Paralytic shellfish toxins (PSTs) are a group of more than a dozen natural seafood contaminants that cannot be destroyed during cooking. Thus, strategies for managing this public health threat rely on monitoring programs whereby harvesting bans are placed on shellfish resources when found to contain toxin levels above the established regulatory action level (typically 0.80 mg STX equivalents kg^{-1} [80 μg STX eq 100 g^{-1}]). The mouse bioassay (MBA), AOAC Method 959.08, remains the formal reference method for making such regulatory decisions (AOAC, 2005a; NSSP, 2007; EFSA, 2009). Advances in chemical methods of analysis, however, have resulted in the development of two LC-FD (liquid chromatography with fluorescence detection) methods that provide alternatives to the MBA. The pre-column oxidation method, AOAC Method 2005.06 (AOAC, 2005b) has been validated and is considered an alternative method to the MBA in certain regulatory environments (EFSA, 2009; EC Regulation, 2006). The post-column oxidation method (PCOX) has undergone single laboratory validation (van de Riet et al., 2009) and is in the final stage of collaborative validation (under the auspices of the AOAC Collaborative Study process). In October 2009, PCOX was accepted as a Type IV NSSP (National Shellfish Sanitation Program) method for the determination of PSTs in shellfish, making it available for regulatory decisions in the US (ISSC, 2009).

Both LC-FD methods take advantage of the ability to oxidize the C4–C12 bond in a complex 3-ring structure characteristic of PSTs, with formation of purine aromatic ring structures which produce a characteristic fluorescence emission (Quilliam et al., 1993). The pre-column oxidation approach involves the oxidation of toxins prior to chromatographic separation (Lawrence et al., 2004, 2005; Turner et al., 2009; Turner et al., 2010), whereas the post-column oxidation method (Oshima, 1995; Thomas et al., 2006; Rourke et al., 2008; van de Riet et al., 2009) separates the toxins on the column and then oxidizes the separated toxins by way of a flow injection process before

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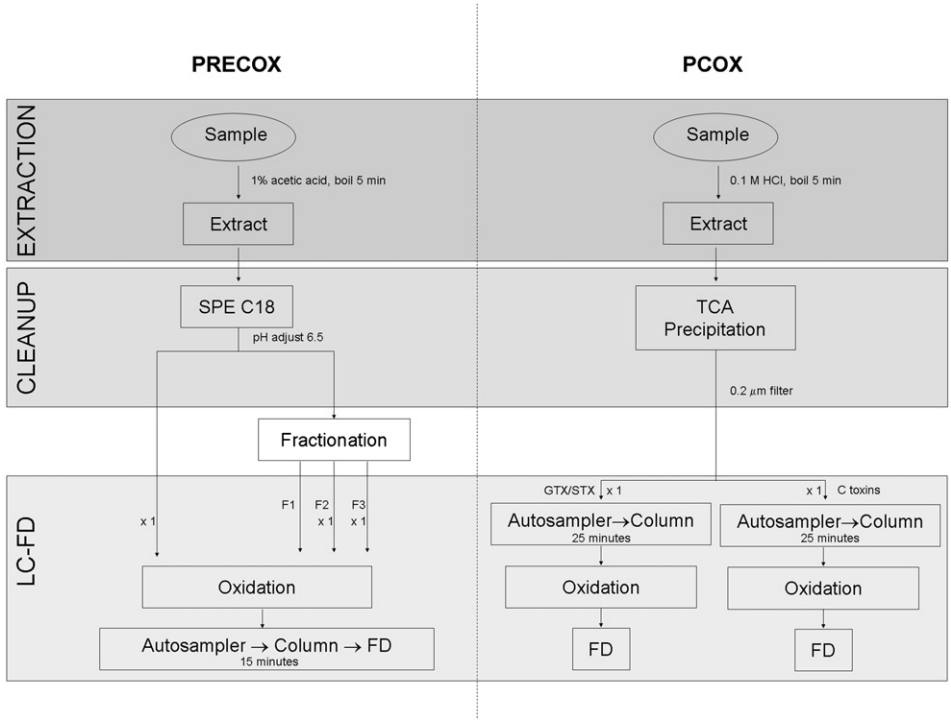

Fig. 1. For each method, 5 g of homogenized shellfish are extracted. Extracts for PRECOX are filtered through an SPE C18 cartridge and pH adjusted to 6.5. One aliquot is oxidized using hydrogen peroxide prior to LC-FD to quantify non-hydroxylated toxins and/or periodate to screen for all toxins. Another aliquot of the pH adjusted, filtered extract is passed through a carboxylic ion exchange cartridge (COOH) to fractionate the toxins into three fractions: (F1) containing the C toxins, (F2) containing the GTXs, and (F3) containing STX, dcSTX, dcNEO and NEO. F1 is oxidized using hydrogen peroxide, whereas F2 and F3 are oxidized using periodate. All oxidized samples are then injected into the LC for analysis using the same gradient conditions for each sample. Extracts for PCOX undergo a TCA precipitation step following by filtration. Filtered samples are then injected into the LC where they are separated on the column and then oxidized in line before flowing through the FD. Chromatographic conditions, including the column, are different for the GTX/STX congeners (Gradient 1) and the C toxins (Gradient 2). Thus, each sample must be analyzed twice to test for the entire suite of toxins.

fluorescence detection (Fig. 1). Like all methods, each has its own advantages and limitations and these are explored in this paper. The LC-FD methods are compared with respect to instrumentation requirements, ease of chromatographic set up, ability to separate individual toxins, data interpretation, expected toxin profiles, sensitivity, comparison to the MBA, method acceptance and implementation.

1.1. Instrumentation requirements

The AOAC 2005.06 pre-column oxidation method (referred to herein as PRECOX) involves a time consuming and rigorous sample preparation. Sample cleanup and toxin fractionation are carried out using C18 solid phase extraction (SPE) and carboxylic acid (COOH) weak ion exchange cartridges, respectively. This type of sample preparation has traditionally been performed manually using vacuum manifolds, making this an onerous task in high sample throughput environments. One of the major complaints about this method and reasons given for the lack of its wider implementation is the length of time required to process samples (Ben-Gigirey and Villar-González, 2008; Etheridge, 2010; Rodríguez et al., 2010). However, the use of automated sample preparation and liquid handling systems allows for semi-automation of these steps (Turner et al., 2009), not only improving speed and efficiency but also enhancing method performance and quality control. At Cefas (Centre for Environment, Fisheries and Aquaculture Science, UK), 40 or more samples a day are regularly processed and analyzed using this technology (A. Turner, pers. comm.). More specifically, 40 samples a day can be screened per instrument with 1 person. During peak season, screening 40 samples per day and assuming ~25% positive, those samples can be quantified in a day with one more instrument and 1 more person. Thus, laboratories with access to such instrumentation can greatly increase the number of samples that can be processed, improving the accuracy and precision and making the method practical for use in routine monitoring programs as compared to the traditional sample preparation with vacuum manifolds. The post-column oxidation method, on the other hand, has a simpler sample preparation procedure, incorporating just protein depletion and a filtration step prior to chromatographic analysis. Therefore, special sample preparation instrumentation is not necessary. However, PCOX requires the capability to deliver reagents by flow injection for oxidation reactions between the chromatographic column and the fluorescence detector, meaning additional LC components are required. Therefore, laboratories that possess post-column delivery systems may consider the PCOX method favorable based on the instrumentation