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Influence of the sample toxic profile on the suitability of a high performance liquid chromatography method for official paralytic shellfish toxins control☆

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Abstract

An HPLC-FLD method, involving pre-chromatographic oxidation of the PSP toxins with hydrogen peroxide and periodate, has been AOAC validated through a collaborative trial and adopted as AOAC Official Method. This method could be a candidate for replacing the mouse bioassay (MBA) for the Official Control of PSP toxins at European level, once accepted by the legislation. An interlaboratory exercise has been organized by the CRLMB to evaluate its "fitness for purpose" for the Official Control of PSP toxins in the EU laboratories. Eighteen EU laboratories took part in the study and had to analyze six bivalve mollusc samples with several PSP toxic profiles. The performance of the participant laboratories in the application of this method was compared with that obtained at the collaborative trial. Information on problems/drawbacks encountered by participants in the application of this method was also sought. The HPLC validated method is only applicable for Official PSP Control for certain samples. This depends on sample PSP toxic profile. Results obtained for samples where only GTX2,3 and STX were present were satisfactory and in agreement with MBA results. Results obtained for a sample with a toxic profile dominated by GTX6 and suspected to contain also C1,2 and C3,4 were not satisfactory. GTX5 and dc-STX could be quantified, although the results achieved (total toxicity) were lower than those obtained by MBA. It can be also useful as a screening method, complementary to MBA, helping in the reduction of the animals used. However, the lack of several PSP standards, the fact that the method is not validated for all the PSP toxins, and several drawbacks found in its application are a handicap to fully implement it for Official PSP Control as a viable replacement for bioassay.

Keywords: Paralytic shellfish poisoning; Toxins; Interlaboratory exercise; HPLC method; Official control

1. Introduction

Paralytic shellfish poisoning is caused by a group of approximately two dozen naturally occurring potent neurotoxins. These toxins specifically block the excitation current in nerve and muscle cells, finally resulting in paralysis and other illness in consumers of contaminated shellfish [1].

Regulation (EC) No. 853/2004 of the European Parliament and of the Council [2], laying down specific hygiene rules for food of animal origin, specifies the required health standards for live bivalve molluscs. This Regulation indicates that live bivalve molluscs placed on the market for human consumption must not contain marine biotoxins in total quantities (measured in the whole body or any part edible separately) that exceed 800 μg/kg for paralytic shellfish poison (PSP).

Commission Regulation (EC) No. 2074/2005 [3] specifies the recognised testing methods for marine biotoxins for the purpose of Regulations (EC) Nos. 853/2004 and 854/2004. This Regulation states that "The paralytic shellfish poison (PSP) content of edible parts of molluscs (the whole body or any part edible separately) must be detected in accordance with the biological testing method or any other internationally recognised method. The biological testing method may be carried out in association, if necessary, with another method for detecting Saxitoxin and any of its analogues for which standards are available. If the results are challenged, the reference method shall be the biological method".

☆ See Appendix A for participants and their respective addresses.

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The mouse bioassay (MBA) for the determination of PSP toxicity was first applied in 1937 to the assay of acidic extracts of mussels. In subsequent years, the general procedure was further standardized and validated in a series of intercomparative studies. This reference method (AOAC Official Method 959.08: Paralytic Shellfish Poison) [4] is internationally recognised for quantifying PSP toxicity and it is used worldwide in PSP monitoring programmes, albeit with some variation in the acceptable regulatory limit for toxicity [5]. In the first meeting of the European Union National Reference Laboratories on Marine Biotoxins (EU NRLs) (Vigo, March 1996) the members of the NRLs agreed that AOAC Official Method 959.08 should be the reference biological testing method.

Regarding the detection of marine biotoxins, Commission Regulation (EC) No. 2074/2005 [3] also mentions that "In addition to biological testing methods, alternative detection methods, such as chemical methods and in vitro assays, should be allowed if it is demonstrated that the performance of the chosen methods is at least as effective as the biological method and that their implementation provides an equivalent level of public health protection". Additionally, it indicates that "Provision should be made for the replacement of biological tests as soon as possible".

Over the past few decades, considerable progress has been made in developing chemical analytical alternatives to the MBA method for almost all the common PSP toxin analogues found in shellfish and other seafood [1]. Among the possible alternatives, Lawrence HPLC method has been AOAC validated through a collaborative trial [6] and recently adopted as First Action 2005.06 AOAC Official Method [7]. This method involves liquid chromatography with fluorescence detection after pre-chromatographic oxidation of the PSP toxins using hydrogen peroxide and periodate. It is applicable to the determination of saxitoxin, neosaxitoxin, gonyautoxins 2 and 3 (combined), gonyautoxins 1 and 4 (combined), decarbamoyl saxitoxin, GTX-5 (B-1), C-1 and C-2 (combined) and C-3 and C-4 (combined) in shellfish (mussels, clams, oysters and scallops). This method could be a candidate for replacing the MBA for the Official Control of PSP toxins at European level, once accepted by the European legislation. For this reason, and in order to evaluate the "fitness for purpose" of the method for the Official Control of PSP toxins in the European laboratories an interlaboratory exercise was organized by the Community Reference Laboratory of Marine Biotoxins (CRLMB).

This paper describes the interlaboratory exercise on paralytic shellfish poisoning toxins determination (Lawrence HPLC method) carried out in 18 EU laboratories at the end of 2005–beginning of 2006.

2. Experimental

2.1. Method and protocol for the exercise

The method used for this exercise was Lawrence HPLC method as appears in First Action 2005.06 AOAC Official Method [7]. The method involves a sample duplicate extraction with acetic acid solution (first extraction with heating) and an extract clean-up step in solid phase extraction (SPE) C18 cartridges. The cleaned extracts are derivatized with periodate and peroxide oxidants prior to analyzing by HPLC with fluorescence detection. Samples containing C1,2 (calibrant not available at this moment), dc-STX, GTX2,3, GTX5, STX and dc-GTX2,3 (although the method is not validated for these last toxins) can be quantified after SPE-C18. Extracts containing GTX1,4, C3,4 (calibrant not available), NEO, and GTX6 (calibrant not available), must be further purified with SPE-COOH cartridges prior to periodate derivatization and HPLC-FLD analysis.

A detailed protocol was prepared by the CRLMB in collaboration with the Dutch National Reference Laboratory (NRL) for this exercise. The protocol facilitated to the participants contained information on the method to be employed and on test materials details, storage and analysis instructions. The protocol for the study and the laboratory code for each participant were sent with the samples.

In order to achieve a uniform procedure in some aspects, instructions/tips for the application of the method for this interlaboratory exercise were included in the protocol. These contained:

A list of *standards needed* for the exercise. Seven PSP standards were selected for being the only certified PSP standards available at that moment. These were: NRC CRM-STX-d, NRC CRM-dc-STX, NRC CRM-GTX 2&3-b, NRC CRM-GTX 1&4-b, NRC CRM-NEO-b, NRC CRM-GTX5-b, NRC CRM-dc-GTX 2&3-b, all of them from the Institute for Marine Biosciences, National Research Council of Canada.

2.1.1. Indications on expression and reporting of the results

Lawrence method [6] indicates the way to calculate the concentration of each PSP toxin (in μg/kg), but it does not indicate how to proceed to report results in total toxicity. In order to express the results in μg equivalents STX dihydrochloride/100 g meat (as previously agreed by the EU NRLs at the VII Meeting of the EU NRLs on Marine Biotoxins, Vigo, November 2004) it was suggested to use the "Supplemental Information for PSP toxin CRMs" that comes with the NRC CRMs [8]. Following these guidelines, the concentration of each PSP toxin is converted into its toxicity contribution (mouse units/litre extract) by using the specific toxicity of a given PSP toxin. Then, the combined toxicity of all PSP toxins (present in a sample) is obtained and transformed into STX equivalent concentration (μg equivalents STX dihydrochloride/100 g meat). Participants had to submit their results in a results proforma facilitated with the protocol, together with information on the PSP toxins detected/quantified.

2.1.2. Toxicity factors for PSP toxins

The specific toxicities (Oshima [9]), corrected as they appear in the "Supplemental Information for PSP toxin CRMs" that comes with the NRC CRMs [8], of the PSP toxins should be used for calculations. For those toxins that are determined together, since they coelute (dc-GTX2 and dc-GTX3; GTX1 and GTX4; GTX2 and GTX3), it was agreed that participants had to choose