LC-UV and LC-MS methods for the determination of domoic acid

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Under European legislation, domoic acid (DA), the main constituent of amnesic shellfish poisoning, is monitored to protect the shellfish consumer. To ensure comparability amongst analytical data, it was deemed necessary to undertake performance assessments of the methods conducted by monitoring laboratories of the United Kingdom and Ireland.

In phase I of a two-phase inter-comparison, three laboratories used high-performance liquid chromatography and ultraviolet detection (HPLC-UV). Concentration data for a DA standard solution, a crude extract of whole scallops and a scallop-homogenate fell within internationally accepted limits, demonstrating good agreement for these matrices. Between-laboratory analyses of a scallop gonad showed a higher variation (>16%).

In phase II, a second gonad homogenate containing DA one order of magnitude higher in concentration gave results acceptable to internationally set criteria.

The efficiency of the strong anion-exchange cartridges used in sample-extract clean-up should be monitored as part of a laboratory quality control system.

From a recovery study, it is suggested that recovery correction should also be applied.

There was no difference in the quantitation of DA in standard solutions or shellfish using either LC-UV or LC with mass spectrometric (MS) detection, and between-laboratory MS data for a gonad homogenate were also equivalent.

Variations of the published method practised by the monitoring laboratories were found not to compromise results, thus demonstrating an acceptable degree of ruggedness, as well as comparability between the participants.

Keywords: Domoic acid; LC-MS; LC-UV; Ruggedness; Scallops; Standard solution

1. Introduction

Amnesic shellfish poisoning (ASP) toxins are produced by microalgae (e.g., *Pseudo-nitzschia* spp.) and can accumulate in shellfish. Consumption of toxic shellfish may cause a number of effects in humans, including vomiting, diarrhea and permanent short-term memory loss [1].

European Union (EU) legislation [2] was amended in 1997 [3], and adopted in the United Kingdom (UK) in 1998, to include domoic acid (DA), the main constituent of ASP toxins, in the suite of biotoxins to be determined in the regulatory monitoring of shellfish. The amendment requires shellfish to contain less than 20 mg/kg of DA as analyzed by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection; however, no specific procedure has been officially validated or recommended by the EU.

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The International Scientific Community has proposed two methods [4,5]. The suitability of these methods is being investigated by the European Committee on Normalization (CEN) and by a EU working group. Irish and UK laboratories involved in the regulatory monitoring of shellfish poisons have since adopted the procedure of Quilliam et al. [4]. A performance assessment was deemed necessary to ensure comparability amongst the monitoring laboratories involved in the routine analyses of ASP, so inter-laboratory comparisons were undertaken involving four laboratories whereby concentration data was reviewed on the basis of applied methodologies.

For the purposes of this inter-comparison study reported here, three biotoxin-monitoring laboratories (LAB-1, LAB-2 and LAB-3) were included along with LAB-4, which participated to develop LC with mass spectrometric (LC-MS) method of analysis. The study consisted of two phases. Thus, this article describes results of an inter-laboratory comparison study, as performed by the four laboratories, of the HPLC-UV and LC-MS methods used for the detection and quantification of DA in standard solutions and in real shellfish samples naturally incurred with DA.

2. Inter-laboratory performance studies

External assessments of the quality of the results generated by individual laboratories and in the form of inter-laboratory comparisons satisfy the requirement to demonstrate comparability of analytical data. By centrally distributing samples, assessments of performance in the inter-laboratory studies were made possible, and the participation in such comparisons proved necessary in method development and refinement, as well as validation.

2.1. Sample management

LAB-1 prepared and distributed all of the standard solutions, scallop-tissue homogenates, and crude extracts used in both phases of this study. A certified reference material [CRM, MUS1-B; National Research Council (NRC) Canada)] containing DA was also examined in phase II. To assess the stability of the samples over the study periods, the DA contents in aliquots of standard solutions, extracts and homogenates were also determined by LAB-1 prior to sample dispatch, and following receipt of the results from each of the participants.

2.2. Statistical analysis and criteria

A comparison of concentration data sets generated by HPLC-UV and LC-MS were evaluated statistically to examine if they were significantly different from each other. This was carried out using a *t*-test or a one-way ANOVA on ranks according to Kruskal–Wallis. Statistical analysis of the data was also conducted in accordance with ''Quality Assurance of Information for Marine Environmental Monitoring'' (QUASIMEME) [6,7], an international proficiency-testing scheme. The scheme assesses the proficiency of participating laboratories by comparing their results with assigned target values. For each analysis, a ''maximum allowable error'' (MAE) [8], is defined as the sum of proportional and constant errors. Data are then assessed as ''satisfactory'', ''questionable'' or ''unsatisfactory'' according to the degree to which they deviate from the assigned or target value. For instance, at a MAE value of 12.5%, data within 25% of the assigned value would be assessed as ''satisfactory''. Target values were established as either the nominal values for standard solutions or the mean of the participant's results for DA content in extracts and tissues.

The statistical model used in this study was based on the QUASIMEME model using proportional errors and did not include any allowance for constant errors. The proportional errors in QUASIMEME inter-comparison exercises are generally between 6% and 12.5%. Since there are no agreed international parameters set for the quality of amnesic shellfish poison (ASP) analyses and, due to the potential implications to public health of DA as a food contaminant, the MAEs set in this study were stricter than those utilized by QUASIMEME. Values also varied depending on the number of sample-processing steps used by the participants during extract preparation. Table 1 details the MAE values assigned for each sample type analyzed in phases I and II of the inter-laboratory exercises.

Table 1. Maximum allowable error (MAE) values for different sample matrices analyzed in the inter-comparison exercises

Sample matrix	MAE (%)	Definition of satisfactory data based on assigned value (%)	Number of processing steps for sample preparation by participants
Standards and crude extract	3.13	6.25	0
Crude extract processed through SAX clean up	4.7	9.38	1
Tissue homogenates[a]	6.25	12.5	2

See text for explanation of MAEs.
[a]Processing steps including extraction and clean up by SAX.