Validation of LC-MS method for determining of lipophilic toxins in shellfish species typically tested in UK

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Background

During periods of cultivation, edible shellfish may become contaminated by naturally occurring toxins derived from certain marine algae. One family of these chemicals is the fat-soluble (lipophilic) toxins. Some lipophilic toxins cause severe gastrointestinal disorders in humans following consumption of shellfish contaminated above regulated levels. To protect the consumer, shellfish monitoring programmes are in place in the United Kingdom to determine the extent of toxicity. Historically, measuring toxicity has been undertaken by a qualitative and non-specific animal test. To reduce reliance on animal tests and to improve risk management of contaminated shellfish, a chemical/analytical method was developed as a suitable alternative.

Research Approach

This involved separation of regulated toxins from complex shellfish extracts coupled to instrumentation for their detection and quantitation.

Toxins were extracted from shellfish flesh using a method published by the EU Reference Laboratory for Marine Biotoxins. A published liquid chromatographic (LC) method was then refined in order to separate 12 EU regulated lipophilic toxins including okadaic acid (OA) and dinophysistoxins (DTX), pectenotoxins (PTX), azaspiracids (AZA) and yessotoxins (YTX) within 23 minutes of analysis. Identification was then confirmed by (tandem) mass spectrometry (MS/MS) detection based on recording ‘fingerprint’ ions specific to each toxin compound. Following international protocols, the performance of the method was established and described after validation and application in EU-wide, inter-laboratory studies.

Results

The refined method provides an efficient, automated, multi-toxin approach which combines an effective extraction procedure with the specificity of LC-MS/MS analysis, allowing for specific identification and more precise quantification of the range of lipophilic toxins encountered in UK commercially significant shellfish species. It demonstrates many advantages over the former EU reference Mouse bioassay (MBA) method, which although is able to respond to the presence of marine lipophilic toxins, can present ‘false’ results (due to interference from co-extracted shellfish matrix compounds) and is not able to provide any quantitative information on toxin concentrations or specifically identify different toxins or toxin compounds.

The refined method proved particularly sensitive for PTX, AZA and YTX toxins and concentrations as low as 3-11% of the EU regulatory limits were achieved satisfactorily from a range of shellfish species commonly produced by the UK's shellfish industry. Sensitivity was limiting for the OA/DTX toxins although much improved compared to the animal assay. For all toxins, an average recovery of 93% was found, reflecting an efficient extraction method for their removal from shellfish matrices. The variability (precision) of the entire method of toxin extraction plus analysis was acceptable. Within a batch of sample analyses, variations of toxin measurements were ~10% or less whereas between batches, variations of up to ~20% were found. Changes made to method parameters did not affect the ruggedness or stability of the method.

During inter-laboratory studies, method performance was found to be comparable with other LC-MS/MS techniques applied in laboratories across Europe that monitor similar marine lipophilic toxins. Valuable information regarding the negative (biasing) effects of shellfish extracts on toxin measurements during sample analysis was gained from these studies. Transferring the method to a new, state-of-the-art, 'fast' LC-MS/MS instrument reduced sample analysis time by 70% ensuring the technique is responsive to meeting the high demands of shellfish monitoring programmes. Additionally, lower toxin concentrations could be confidently determined. With the advantages of high sensitivity, toxin identification, confirmation and quantitation, the validated method is practical and fit-for-purpose. It provides a suitable replacement for the animal assay and recommendations are made for its implementation in the UK biotoxin statutory monitoring programmes.

Research report

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