The event of quality control non conformances biology essay

Science, Biology

The purpose of this Test method is to detail the procedure for the determination of Amnesic Shellfish Poisoning (ASP) toxicity (caused by domoic acid-DA) in molluscan shellfish by high performance liquid chromatography with ultraviolet detection (HPLC- UV). Consumption of contaminated shellfish can lead to intestinal distress. Severe poisoning can cause a facial grimace or chewing motion, short-term memory loss and difficulty breathing.

Scope and Application

This method is applicable to molluscan shellfish in the live, frozen or processed state. It is both reproducible and accurate.

Specificity

The specificity of this chemical method is high. The extraction with methanol: water ensures specificity. While the sample clean up with strong anion exchange (SAX) solid phase extraction (SPE) allows for trace level detection (Quilliam et al, 2005).

Principle

Domoic acid (DA) analysis is based on the High Performance Liquid Chromatography with (HPLC-UV) method by Quilliam et al. (1995). A 50% methanol solution is used to extract DA in a homogenised shellfish sample. It is followed by a SAX-SPE sample clean up. This extract is then analysed using isocratic LC with Photo diode array (PDA) and the DA content is determined using UV detection (at 242nm). The results are quantified using Certified Reference materials (CRMs) of DA.

Responsibility

It is the responsibility of the Scientist to ensure that all staff members (technicians and assistant technicians) are trained and the test method is implemented and adhered to, by all staff members. It is the responsibility of all laboratory staff members to comply with this test method and to notify the Laboratory scientist immediately if difficulties are experienced.

Procedure

Safety:

3. 1. 1Ensure that rubber gloves are used when handling materials, which may contain DA. 3. 1. 2Use Nitrile gloves when handling Acetonitrile and methanol. 3. 1. 3All solvent work with trifuoroacetic should be carried out under fume hood. 3. 1. 4Please refer to and familiarise yourself with Material Safety Data Sheets before using reagents in the analysis.

Apparatus and Materials:

3. 2. 1Glass measuring cylinders3. 2. 2Glass beakers, various sizes3. 2. 3Graduated cylinders various sizes3. 2. 4Weighing boats3. 2. 5Shucking knife3. 2. 6Glass funnel3. 2. 6No. 10 sieve3. 2. 8Graduated centrifuge tube3. 2. 9Analytical balance (to 0. 1 decimal place) calibrated and verified (as per procedure SOP/G/03) to cover the daily working range of the balance. 3. 2. 10Electric Warring blender with suitable sized goblets, which ensure high homogenisation of sample3. 2. 11Analytical balance (to 0. 01 decimal place) calibrated and verified (as per procedure SOP/G/03) to cover the daily working range of the balance3. 2. 12Plastic spatulas3. 2. 13Strong anion exchange (SAX) cartridges containing 500mg of silica derivatized with

quaternary ammonium silane3. 2. 14Automatic Pipette (0. 00-1000µl) with 0. 01 ml increments verified and used according to SOP/G/053. 2. 15Parafilm3. 2. 16Ultraturrax with 8mm diameter shaft headKimwipesHPLC with C18 column (25cm long x4. 6 mm i. d packed with 5-10 um bonded silica gel with guard column)Fridge & Freezer for storage (calibrated and verified as per SOP/B/51)

Reagents and Materials

3. 3. 1Acetonitrile HPLC Grade (99% purity)3. 3. 2Methanol HPLC Grade (99% purity)Formic acid analytical gradeTrifluoroacetic acid (TFA 99% purity)Citric buffer eluent (Prepared according to SOP/B/02)Deionised water (HPLC grade)Domoic acid working solutions made up from certified calibration standards according to SOP/B/05HPLC mobile phase (prepared according to SOP/B/01)Certified ASP mussel reference material (Quality control sample)

3. 4Sample Receipt

3. 4. 1 The sample should arrive complete with official documentation to indicate origin and sample date (see F/B/30 sample request form). Shellfish samples arriving without this documentation may be rejected (Section 3. 5). 3. 4. 2The details in the sample request form should be entered into the sample receipt log book by authorised personnel, were it is assigned a specific unique Test Identifying Code (TIC). This Test Identifying Code is to be recorded on all vessels/containers used to carry out the analysis throughout the whole procedure. The sample receipt form (F/B/21) is also filled in. 3. 4. 3When shellfish arrive late in the day, i. e. a full analysis is not possible, the sample may be stored either whole or homogenised by refrigeration at 4 ℃ 3

℡C, or may also be frozen below -20ºC overnight or over the weekend. 3. 4. 4The flesh from two species of shellfish are tested, Pacific Oyster (Crassostrea gigas), European flat Oyster (Ostrea edulis). Phytoplankton filter samples and mussels may also be tested when required. 3. 4. 5All samples received by the laboratory should be on ice and attempted for analysis. 3. 4. 6If the shellfish show signs of decay, they may also be rejected, pending a decision by the Scientist. 3. 5Rejection ProcedureIf a sample is rejected, the accompanying sample request form should be completed (with the reason for rejection) and communicated to the Namibian Standards Institution (NSI) Fishery Inspectorate immediately and filed accordingly. On receipt, the Fishery Inspectorate will arrange a second sample as appropriate to be taken from the area to be dispatched to the laboratory as soon as possible.

Extraction

Accurately weigh out 4. 0 + 0. 1g of the tissue homogenate (prepared as per SOP/B/57) into a 50ml graduated centrifuge tube labelled with the TIC and fill in form F/B/23. Add 16. 0 ml of the extraction solvent (prepared as per SOP/B/03). Homogenise the mixture using an Ultraturrax at medium speed for about one minute leaving the tissue remaining on the homogeniser probe. Centrifuge the sample at 3000 rpm for 10 minutes at about 4 oC. Condition SAX cartridge by passing 6ml Methanol, then 3ml water followed by 3ml of the extraction solvent. Drops should flow at about one drop per second. Do not allow cartridge to go dry during this process. Load 5. 0 ml of the filtered supernatant onto the cartridge and allow drops to fall at about 1 drop per second. Stop flow when sample meniscus reaches the top of the

cartridge pack. Discard effluentAdd 5ml of cartridge wash solution (made up of 1: 9 of acetonitrile to water)Allow drops to flow at one drop per second. Stop the flow when sample meniscus reaches the top of the cartridge pack. Discard effluentAdd 0. 5 ml citrate buffer eluent (SOP/B/02). Allow to flow and stop flow when eluent meniscus reaches the top of the cartridge pack. Discard effluent. Elute Domoic acid by using 2ml citrate buffer eluent. Collect this eluent in a centrifuge tube. Stop flow when the 2ml mark is just reached (i. e. on centrifuge tube). Mix solution and withdraw an aliquot for HPLC analysis. Samples should only be stored in the refrigerator for one week in a well sealed screwed capped glass vial.

Note: Do not freeze the SAX eluent extraction as the DA will decompose under these conditions.

3. 7HPLC Determination:

Prepare the HPLC machine as per SOP/B/56 before analysisMake up dilutions of 0. 20, 0. 50, 1. 0, 2. 0, 5. 0 and 10. 0 µgml-1 of CRM DA using SOP/B/05The HPLC should be run with a 250 x 4. 6mm C18 LC column. An isocratic system will be used for the mobile phase with a flow rate of 1. 0 – 1. 5 ml/minute with a column temperature of 40 oCInject sample extracts and standard dilutions (20µl) in duplicate to obtain an average peak area. Samples should be run in the following order: reagent blank, working CRM’s, reagent blank, 10 samples, reagent blank. The reagent blank should be run after every ten samples. The QC sample is treated as a sample in this instanceUse the peak average to determine the concentration of DA in the standards and use this in the determination of the unknown samples.

Calculation is detailed below. The ASP HPLC toxin analysis form F/B/23 should be completed accordingly.

3. 8Calculations

3. 8. 1The concentration of DA (mg kg-1) is calculated using the formula bellow. 3. 8. 2Concentration of DA (mg kg-1) = (As/Ac) (Cc/W) (F). Where: As = average peak area of sampleAc = average peak area of calibration standardsCc = standards concentrationW = weight of tissue homogenate extractF = dilution factorNote: F= 8 for SAX cleaned samples and F= 100 for crude extracts

3. 9Results

Results from the HPLC run are analysed and the concentrations of DA in the sample is calculated and recorded in form F/B/23

3. 10Toxicity Evaluation

The regulatory limit for DA in shellfish is 20mg/kg flesh. Thus any values exceeding this are considered dangerous, thus the sample is not fit for human consumption.

3. 11Quality Control

3. 11. 1Standards are run with unknown samples at all times. 3. 11. 2A sample reagent (control) is also generated by taking it through the whole extraction process (as per section 3. 7 of this Test method)3. 11. 3Form F/B/36 should be completed and the acceptable r2 should be 0. 9999. 3. 11. 4A quality control sample of the certified reference material is run with each sample. 3. 11. 5If a peak, within ± 0. 5minutes from the average retention

time of the calibration standards of the run, is present in the reagent or sample blank then the batch must be rejected and reanalysed.

3. 12Quality Control Results

The results from the HPLC- UV analysis are calculated for each sample to determine the concentration of DA. The QC sample's concentration is plotted into a QC chart.

3. 13Actions in the event of Quality Control non-conformances

3. 13. 1In the event of values higher that the regulatory limit (20mg/kg flesh), the laboratory Scientist should be consulted for appropriate action. 3. 13. 2If the QC falls out of it's specified range (33. 15-47. 15 mg/kg) , a new QC should be made up and analysed accordingly.

Sample receipt form (F/B/21)

Once a sample meets the full acceptance criteria for analysis, F/B/21 is generated. The assigned TIC for the sample is recorded with the date. Shucking step records the date of shucking, the analyst responsible, the weight of the blended tissue, the balance used, fridge/freezer ID (if the sample has to be stored where a full analysis is not possible for that date) and the analyst responsible for conducting these parts. To ensure anonymity between the analyst and the sample under analysis, the sample details must only be completed once all of the above parameters and aspects have been completed and recorded3. 14. 4 Sample details are transcribed from form F/B/30 (sample request form), and record the following details: Sample

location, sample point code and sender detailsDate and type of sample takenReceipt date and time in the lab

4. Reporting Results

4. 1Reporting Results to the Fishery Inspectorate and clients

Results are reported via fax as a certificate of analysis to the farmer and the shellfish inspector. Before faxing the results, Form F/B/21 should be fully completed and verified to include all technical aspects and calculations which relate to the sample tested. Once verified and dated, the report date and time should be completed immediately prior to faxing. For the purpose of complying with EU and International requirements it is required that no more than three days should elapse from receipt of samples to issuing of results.