Draft Tanzania Standard

Determination of Methylmercury in fish and shellfish

TANZANIA BUREAU OF STANDARDS

Determination of Methylmercury in fish and shellfish

0. FOREWORD

This draft Tanzania standard prescribes the chromatographic method for the determination of methylmercury in fish and shellfish.

In the preparation of this Tanzania standard assistance was derived from AOAC 983.20 Mercury (methyl) in fish and shellfish.

In reporting the result of a test or analysis made in accordance with this standard, if the final value observed or calculated, is to be rounded off, it shall be done in accordance with TZS 4(See clause 2)

1.0 SCOPE

This draft Tanzania standard specifies the chromatographic method for the determination of methylmercury in fish and shellfish.

2.0 REFERENCES

The following referenced standards are indispensable for the application of this standards. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced standard (including any amendments) applies;

TZS 4, Rounding off numerical values
TZS 59, Water - Distilled quality – Specification

3.0 PRINCIPLE

Organic interferences are removed from homogenized material by acetone wash followed by benzene wash. Protein-bound methyl Hg is released by addition of HCl and extracted into benzene. Benzene extract is concentrated and analyzed for CH_3HgCl by GC.

4.0 APPARATUS

4.1 Glassware

Wash all glassware with detergent and rinse thoroughly with hot tap water followed by distilled or deionized water.

4.2 Centrifuge

Model UV or equivalent.

4.3 Centrifuge tubes

50 mL capacity with ground glass or Teflon-lined stoppers.

4.4 Kuderna-Danish (K-D) concentrators

250 mL flask and 10 mL graduated concentrator tube

4.5 Snyder distilling column

Use No. 503100 size 0003 (Kontes Glass Co.) as is or modify Kontes No. 503000, size 121, in either

of 2 ways:

4.5.1 Shorten 3-section, 3-ball column to 2-section, 2-ball column by cutting off top at uppermost constriction.

4.5.2 Insulate 3-section, 3-ball column by wrapping glass wool around top section and holding it in place with AIluminium foil. Glass wool and foil must surround only top section above top ball.

4.6 Carborundum boiling chips

20 mesh, HCI-washed.

4.7 Graduated cylinders

Class A, 25 mL capacity, with ground-glass stopper.

4.8 Transfer pipettes

Disposable glass, Pasteur-type 53/4 in (15 cm) long,

4.9 Dropping pipets

5 mL capacity (No. l3-7IOB, Fisher Scientific Co or equivalent).

4.10 Gas chromatograph

Hewlett Packard Model 57l0A (replaced by HP-5890 series II) or equivalent, equiped with linear ^{63}Ni electron capture detector and 6 ft (1.8 m) x 2 mm id silanized glass column packed with 5% DEGS-PS on 100-120 mesh Supelcoport (Supelco, Inc., No. 1-1870). Pack column no closer than 2.0 cm from injection and detector port nuts and hold packing in place with 2 cm high quality, silanized glass wool at both ends. Install oxygen scrubber and molecular sieve dryer (No. HGC-145, Analabs, Inc. or equivalent) between carrier gas supply and column. Condition column according to manufacturer's instructions as follows: Flush column for 0.5 h with carrier gas flowing at 30 mL/min at room temperature, Then heat for l h at 100° C. Next, heat column to 200°C at programmed heating rate of 4°C/min and hold at 200°C overnight. *Do not connect column to detector during this conditioning process.* Maintain 30 mL/min carrier gas flow at all times during conditioning, treatment, and use. Operating conditions: column 155°C; injector 200°C; detector 300°C; carrier gas flow 30 mL/min; and recorder chart speed 0.5-1.0 cm/min. Under these conditions and with $HgCl_2$ column treatment procedure described below, CH_3HgCl peak will appear 2-3 min after injection.

5.0 REAGENTS

5.1 Solvents.

Acetone, benzene, and isopropanol are all distilled in glass (Note: Benzene is a possible carcinogen.)

5.2 Hydrochloric acid solution - (1:1)

Add concentrated HCI to equal volume of distilled or deionized water and mix. Extract HCI solution 5 times with ¼ its volume of benzene by shaking vigorously 15 s in separator. Discard benzene extracts. Solution may be mixed in advance but must be extracted immediately before use.

5.3 Carrier gas

GC quality Ar-CH_4 (95: 5).

5.4 Sodium sulfate

Heat overnight in 600°C furnace, cool and store in capped brown bottle. Line cap with Aluminum foil to

prevent contamination from cap.

5.5 Methyl mercuric chloride standard solutions
Keep tightly stoppered.

5.5.1 Stock standard solution
1000 µg Hg/mL weigh 0.1252 g CH_3HgCl into 100 mL volumetric flask. Dilute to volume with benzene.

5.5.2 High intermediate standard solution
40 µg Hg/mL Dilute 10.0 mL stock solution to 250.0 mL with benzene.

5.5.3 Low intermediate standard solution
2.0 µg Hg/mL Dilute 10.0 mL high intermediate standard solution to 200.0 mL with benzene.

5.5.4 Working standard solutions
0.010--0.30 µg Hg/mL Prepare monthly by diluting with benzene in volumetric flasks as follows: Dilute 15 mL of 2.0 µg Hg/mL standard to 100.0 mL, 10.0 mL to 100.0 mL, and 10.0 mL to 200.0 mL for 0.30, 0.20, and 0.10 g Hg/mL, respectively. Dilute 20 mL of 0.1 0 µg Hg/mL standard to 25.0 mL, 10.0 mL to 25.0 mL, 10.0 mL to 50.0 mL, and 10.0 mL to 100.0 mL for 0.080, 0.040, 0.020, and 0.010 µg Hg/mL, respectively.

5.6 Mercuric chloride column treatment solution
1000 ppm (µg/mL) $HgCI_2$.Dissolve 0.1 g $HgCl_2$ in 100 mL benzene.

6.0 MERCURIC CHLORIDE COLUMN TREATMENT
5% DEGS-PS conditioned according to manufacturer's instructions can be used to determine CH_3HgCl only after treatment by $HgCl_2$ solution, 5.6. Treat column any time column has been heated to 200°C. Because column performance degrades with time, also treat column periodically during use. Perform appropriate $HgCl_2$ treatment procedures described below. Procedure (6.2) produces most stable baseline and is recommended over procedure (6.3) for routine use.

6.1 Following 200°C column conditioning:
If column has just been conditioned overnight at 200°C, use this procedure. Adjust column temperature to 160°C and connect detector. When baseline is steady treat column by injecting 20 µL $HgCI_2$ treatment solution 5 times at 5 - 10 min. intervals. (Change in column performance may be monitored by injecting 5 µL 0.0 I 0 µg Hg/mL standard solution before and between $HgCl_2$ treatment solution injections.) During treatment procedure, large broad peaks will elute. (CH_3HgCl peak retention time will decrease and peak height will increase.) Approximately 11/2 – 13/4 h after last $HgCI_2$ treatment solution injection, a final large peak will elute. (CH_3HgCl peak height and retention time will be stable.) This broad peak and CH_3HgCl peak height stability signal completion of treatment process. Adjust column temperature to 155°C and wait for steady baseline; then column is ready for use.

6.2 One day preceding extract analysis:
If column has been treated by procedure (6.1) or used at 155°C to analyze test extracts, column may be treated at end of working day for next day's use as follows: Lower column temperature to 115°C and inject 20 µL $HgCI_2$ treatment solution one time. Broad peaks will elute between 11 and 15 h after $HgCl_2$ injection. Next working day, increase column temperature to operating temperature. When baseline is steady (about 15-30 min), column is ready for use.

6.3 During extract analysis at 155°C:
If column has been used at 155°C for extract analysis and column performance has degraded enough

to require $HgCl_2$ treatment, increase column temperature to 160°C, inject one 20 µL aliquot of $HgCl_2$ treatment solution, and monitor baseline. Large, broad peaks will elute I-I1/2h after $HgCl_2$ injection, signaling completion of treatment process. Decrease column temperature to 155°C and wait for steady baseline; then column is ready for use.

7.0 EXTRACTION OF METHYL MERCURY CHLORIDE

Perform all operations, except weighing, in laboratory hood.

7.1 Accurately weigh 2 g homogenized test portion into 50 mL centrifuge tube. Add 25 mL acetone, stopper, and shake vigorously . Remove stopper, cover with foil, and centrifuge 2-5 min at 2000 rpm. Carefully decant and discard acetone. (Use dropping pipet to remove acetone, if necessary.) Repeat 25 mL acetone wash step twice more. Break up tissue with glass stirring rod before shaking, if necessary. Add 20 mL benzene, stopper, and shake vigorously 30 s. Remove stopper, cover with foil, and centrifuge 2-5 min at 2000 rpm. Carefully decant (or draw off with dropping pipet) and discard benzene. Extraneous peaks in final GC analytical chromatograms indicate that more vigorous shaking with acetone and benzene is required.

7.2 Add 10 mL HCI solution to centrifuge tube containing acetone and benzene-washed test portion. Break up tissue with glass stirring rod, and extract tissue by adding 20 mL benzene and shaking gently but thoroughly 2 min. Remove stopper, cover with foil, and centrifuge 5 min at 2000 rpm. If emulsion forms, add 2 mL isopropanol and gently stir benzene layer to break emulsion, taking care not to disturb aqueous phase, and centrifuge. Carefully, transfer benzene layer to K-D concentrator, using 5 mL dropping pipet. Rinse centrifuge tube walls with 3-4 mL benzene and transfer rinse to K-D concentrator. Repeat extraction step twice more, adding 20 mL benzene and shaking I min each time. Combine all 3 benzene extracts in K-D concentrator.

7.3 Place 4-6 boiling chips in K-D concentrator, connect Snyder column, wet Snyder column bubble chambers with 3-4 drops of benzene, and immediately place tube in steam bath or vigorously boiling water bath. Evaporate so that 8 mL remains when cooled to room temperature. Cool. Disconnect concentrator tube and quantitatively transfer solution to 25 mL glass-stoppered graduate using Pasteur-type transfer pipet. Dilute to 20.0 mL with benzene and mix. Add 4 g Na_2S0_4 and mix again. Na_2S04 must be added to 20 mL concentrated extract within 10 h of first acetone wash. Tightly stoppered extracts may be held overnight at this point. Analyze by GC.

8.0 CHROMATOGRAPHY

Verify that system is operating properly by injecting 5 µL volumes of 0.01 µg Hg/mL working standard solution into chromatograph. Difference between CH_3HgCI peak heights for 2 injections should be ≤4%. Check detector linearity by chromatographing all 0.01-0.30 µg Hg/mL working standard solutions.

Inject duplicate 5 µL volumes (equivalent to 0.5 mg test portion) of extract. Difference between CH_3 HgCI peak heights for 2 injections should be ≤4%. Next, inject duplicate 5 µL volumes of standard solution with CH_3HgCl concentration approximately equal to or slightly greater than extract CH_3HgCl concentration. Because column performance and peak height slowly decrease with time, calculate each extract concentration by comparison to standard solution injected immediately after extract.

Calculate methyl Hg content of homogenate in µg Hg/g (ppm Hg) by comparing average CH_3HgCl peak height of duplicate test injections with average CH_3HgCl peak height of duplicate standard injections.

µg Hg/g (ppm) = (*R/R') x (C'/C*) x 20

Where
R = average peak height of duplicate test solution injections;
R' = average peak height of duplicate standard injections;
C = g test portion;
C' = concentration of Hg in CH_3HgCl standard solution (µg Hg/mL).