

**ORTEP STABILIZER TASK FORCE (STF) ANALYTICAL METHOD -
EXTRACTION OF ALKYL TIN CHLORIDES FROM DRY ANIMAL FEED**

SUMMARY

Dry feed samples are spiked with a multi-component internal standard and mixed with methanol and a pH 4.5 buffer. Mono-, di-, and trialkyl (methyl, butyl, or octyl) tin chlorides are simultaneously derivatized and extracted using sodium tetraethylborate and hexane. The derivatized extracts are dried over sodium sulfate and its volume is reduced to 1 mL. An aliquot of each is then transferred to an autosampler vial for analysis with gas chromatography.

GENERAL INFORMATION

--This method is intended for use in conjunction with the corresponding ORTEP Association (2006) method, *Gas Chromatographic Analysis of Derivatized Alkyltin Chlorides*.

--Reagents may contain organotin impurities. It is essential to verify the purity of reagents before use, and monitor contamination by analyzing reagent blanks with each analytical batch.

--Reference samples should be prepared in the matrix that is being extracted. For this extraction, the reference spike solutions should be added to corn oil so they can be mixed with the appropriate amount of feed material and included in the extraction.

--When pre-treating samples and calibration standards, an internal standard is needed for each degree of alkylation (i.e., the internal standard solution should contain a mono-, di-, tri-, and tetra-substituted compound).

--While preparing the German Standard method (DIN 38407 F13), a round robin test was performed and significant singular deviations were observed. Therefore, it is mandatory that two independent determinations of each sample be performed.

I. Reagents and Solutions

A. Chemicals

Acetic acid, CH₃COOH (glacial), 99+%

Acetone, (CH₃)₂CO, HPLC Grade

Hexane, C₆H₁₄, HPLC Grade

Hydrochloric acid, HCl, 2 M

Methanol, CH₃OH, HPLC Grade

Naphthalene, C₁₀H₈, 98+%

Sodium acetate-Acetic acid buffer, pH 4.5

Sodium sulfate, Na₂SO₄ (anhydrous)

Sodium tetraethylborate (STEB), NaB(C₂H₅)₄ – **Caution: STEB may spontaneously combust in air! Use appropriate safety measures when**

ORGANOTIN ENVIRONMENTAL PROGRAMME



handling.

Monomethyltin trichloride (MMTC), CH_3SnCl_3

Dimethyltin dichloride (DMTC), $(\text{CH}_3)_2\text{SnCl}_2$

Trimethyltin chloride (TMTC), $(\text{CH}_3)_3\text{SnCl}$

Monobutyltin trichloride (MBTC), $\text{C}_4\text{H}_9\text{SnCl}_3$

Dibutyltin dichloride (DBTC), $(\text{C}_4\text{H}_9)_2\text{SnCl}_2$

Tributyltin chloride (TBTC), $(\text{C}_4\text{H}_9)_3\text{SnCl}$

Monooctyltin trichloride (MOTC), $\text{C}_8\text{H}_{17}\text{SnCl}_3$

Dioctyltin dichloride (DOTC), $(\text{C}_8\text{H}_{17})_2\text{SnCl}_2$

Trioctyltin chloride (TOTC), $(\text{C}_8\text{H}_{17})_3\text{SnCl}$

Monoheptyltin trichloride (MHTC), $\text{C}_7\text{H}_{15}\text{SnCl}_3$ (internal standard)

Diheptyltin dichloride (DHTC), $(\text{C}_7\text{H}_{15})_2\text{SnCl}_2$ (internal standard)

Tripropyltin chloride (TPTC), $(\text{C}_3\text{H}_7)_3\text{SnCl}$ (internal standard)

Tetrapropyltin (TTPT), $(\text{C}_3\text{H}_7)_4\text{Sn}$ (internal standard)

B. Prepared Reagents and Solutions

1. Stock Solution A (Multi-component Standard Solution): Prepare a 1 mg/mL organotin cation stock solution by weighing the organotin compounds of interest according to Table 1 (to an accuracy of ± 0.1 mg) into a tared 100 mL volumetric flask. Dissolve in a small amount of methanol, make up to volume with methanol, and homogenize.

NOTE: All standards should be labeled with actual concentrations (vs. theoretical). For example, if theoretically using 0.1 g/100 mL (0.001 g/mL), but actually used 0.11 g/100 mL (0.0011 g/mL), the solution should be labeled with the actual concentration of 0.0011 g/mL.



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Table 1. Masses of organotin compounds required for stock solutions A and B (@ 1.0 mg/mL) and weighing factors for recalculation to organotin cations (assuming 100% purity of the substances)

Substance	Weighing Factor*	Mass** (mg)	Solution
Monomethyltin trichloride	0.557	179.5	A
Dimethyltin dichloride	0.677	147.7	A
Trimethyltin chloride	0.822	121.6	A
Monobutyltin trichloride	0.623	160.5	A
Dibutyltin dichloride	0.767	130.4	A
Tributyltin chloride	0.891	112.2	A
Monooctyltin trichloride	0.686	145.8	A
Diocetyl tin dichloride	0.830	120.5	A
Triocetyl tin chloride	0.928	107.7	A

Monoheptyltin trichloride	0.672	148.8	B
Diheptyltin dichloride	0.817	122.4	B
Tripropyltin chloride	0.875	114.3	B
Tetrapropyltin	1.000	100.0	B

* - Weighing factor = molar mass organotin cation/ molar mass organotin compound

** - In case of deviating masses, the actual concentration of the OTC is calculated using the weighing factor

Solution A = The multi-component stock standard solution (see Section I.B.1)

Solution B = The internal standard stock solution (see Section I.B.2)

2. **Stock Solution B (Internal Standards):** Prepare a 1 mg/mL internal standard stock solution by weighing the appropriate propyltin and heptyltin compounds according to Table 1 (to an accuracy of ± 0.1 mg) in a tared 100 mL volumetric flask. Dissolve in a small amount of methanol, bring to volume with methanol, and homogenize.

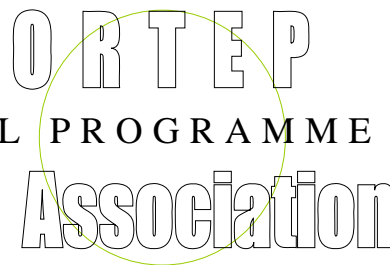
NOTE: As per Table 1, for the preparation of exactly 1 mg/ml of organotin cation internal standards, 148.8 mg of monoheptyltin trichloride, 122.4 mg of diheptyltin dichloride, 114.3 mg of tripropyltin chloride and 100.0 mg of tetrapropyltin are necessary.

3. **Derivatization Agent:** Prepare a solution containing 20% (w/v) of STEB in reagent water.

NOTE: This solution is stable for at least three months if it is stored under inert gas, at 4 °C, and out of direct light.

4. **Sodium Acetate-Acetic Acid Buffer, pH 4.5:** Dissolve about 82 g of sodium acetate in 500 ml of water in a 1-L volumetric flask. Add sufficient glacial acetic acid to reach a pH of 4.5. Make up to volume with water and homogenize.

ORGANOTIN ENVIRONMENTAL PROGRAMME



5. Drying Agent: Add approximately 100 g of granular sodium sulfate to a quartz plate and dry in a 180°C oven for at least 4 hours. Pour the dried sodium sulfate into a wide-necked bottle, let cool to room temperature, and close tightly.

C. QA/QC Samples

1. Reference Solution Samples (Dry Feed Multi-component Reference Standards):

Using the same mass of feed as the samples, prepare at least 6 reference standard samples by spiking with the reference standard stock solution described in Section I.B.1. The standard solution should be added into corn oil and then mixed with the feed sample. Spiking should occur at (approximately) equidistant concentration levels surrounding the anticipated sample analyte concentrations (e.g., if expected sample concentration = 10 µg/g, then reference standards could be spiked at 1, 5, 10, 15, 20 and 25 µg/g). A calibration range greater than one order of magnitude can be used, if linearity and repeatability can be demonstrated and only the upper 60–70 % of the calibration curve is used. The lower end is adversely affected due to relatively high variation. Calibration points by factors (e.g. 0.1, 1, 10, 100) are not acceptable. In order to accomplish an appropriate precision, two calibrations should be performed: one covering the lower working range, and one covering the upper working range. (E.g., working range is 20–500 µg/g; therefore, the final extract concentration is 40–1000 µg/ml. Divide in terms of final extract concentrations into lower working range 40–240 µg/ml and upper working range 250–1000 µg/ml. The internal standard concentration in the final extract should then be 100 µg/mL).

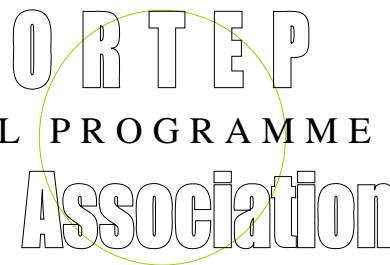
NOTE: For calculation of the amount of monoalkyl tin compounds, the peak area of MHTC is used as the internal standard. For calculation of the amount of dialkyl tin compounds, the peak area of DHTC is used. For calculation of the amount of tri- and tetraalkyl tin compounds, the peak areas of TPTC and TTPT, respectively, are used.

2. Method Blank: For each analytical batch prepare a method blank of rat feed at the same mass as the samples being analyzed.
3. Matrix Spikes: For each analytical batch prepare a matrix spike and a matrix spike duplicate using the same procedure, only using sodium sulfate instead. Spike the samples with the compounds of interest at the approximate concentrations expected in the samples. A laboratory control spike (LCS) or “blank spike” can also be prepared using the same procedure, only using sodium sulfate instead of actual sample.

II. Apparatus

- A. GC autosampler vials, 2 mL (amber-colored, with PTFE-lined septum hole caps)
Mechanical shaker
Muffle furnace (up to 500°C ± 20°C), optional

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Oven (up to 200°C)

Quartz plate, 12 cm diameter

Sample containers - 50 mL Corning tubes

Solvent evaporation apparatus (e.g., N-Evap, Turbovap, Rotary evaporator, etc.)

Transfer pipettes (disposable)

Volumetric flasks (10, 50, 100, and 1000 mL)

Volumetric pipettes (varied volumes)

- B. Glassware: Use of PTFE, polycarbonate, or some form of disposable plastic containers/apparatus is an option, as methyltins have been shown to sorb to glass. Prior to using plastic containers however, verification that they do not leach tin at levels greater than analysis detection limits should be obtained (e.g., a leaching study should be performed, perhaps using total tin analysis).

Glassware cleaning of non-disposable apparatus should be performed after each analysis, and should include washing with a laboratory cleaner (e.g., Alconox™), followed by repeated rinsing with reagent water, acetone, and hexane prior to initiation of the next round of analyses. Examination of all non-disposable glassware for cross-contamination should be conducted frequently. If a batch of glassware is suspected of contamination due to exposure to high organotin concentrations, it is advisable to acid-rinse all glassware with high purity, 10 N nitric acid or equivalent. Acid rinsing removes even ultra-trace quantities of organotins adhering to glass surfaces.

III. Feed Extraction Procedure

1. Samples should be stored in pre-cleaned sample containers at 4°C for a holding time of no more than 24 hours. Samples should be frozen if storage is to be prolonged. However, holding time studies may be performed to evaluate this issue further.
2. Weigh out 2 g of sample into a sample container. Note the actual mass used in order to calculate the final results. Prepare the necessary QC samples as appropriate (i.e., reference standards, method blank, matrix spike, matrix spike duplicate – see Section I.C.).
3. Treat all samples, blanks, QC spikes, and reference standards as follows:
 - a) Add an appropriate amount of internal standard solution (see Sections I.B.2 and I.C.1).
 - b) Add 5.0 mL methanol.
 - c) Add 5.0 mL acetate buffer.
 - d) Add 2.0 mL derivatization solution (20% aqueous STEB).

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- e) Add 5.0 mL hexane (containing ~0.1 mg/L naphthalene to check the injection of the sample into the GC instrument). Cap each container and shake for 15 minutes (intensity ~250 rpm).

NOTE: Method validation study also evaluated using 10.0 mL methanol (step b) and 10.0 mL hexane (step e); recovery effects varied by test substance.

- f) Place all sample containers in an oven at approximately 60°C for 15 minutes.
- g) Allow the phases to separate, remove approximately 3 mL of the hexane (upper) layer and wash it with approximately 3 mL of 2 M HCl (shake for 30 minutes at 250 rpm).
- h) Transfer an aliquot of the hexane top layer into an amber-colored GC autosampler vial and store at 4°C, in the dark, until analysis. Extracts may be stored for at least 2 weeks. Depending on the concentrations of the calibration and analytical samples, dilution of the extracts may be required prior to analysis. Dilutions should be performed using hexane (containing ~0.1 mg/L naphthalene).

IV. Extract Cleanup

Depending on the source of the samples being analyzed, extract cleanup may be required prior to analysis (make sure that calibration and QC samples are treated accordingly). Commercially pre-packed silica gel columns may be used or cleanup columns may be prepared using the following steps. Both commercial and lab-packed columns should be tested for contamination prior to use, and to ensure that appropriate recoveries can be achieved.

(Please note, this procedure was not attempted during the method validation study)

1. Add approximately 120 g of silica (grain size 63–200 nm) to a quartz plate and heat in a muffle furnace at 500°C (\pm 20°C) for at least 12 hours. Make sure that the temperature does not exceed 520°C. Let the plate cool in the oven to about 200°C, pour the silica gel into a wide-necked glass bottle, and let cool to room temperature. Add water equal to 3% of the silica gel mass, to the cooled silica gel. Stopper the bottle and homogenize the contents for 2 hours on a shaker.

Check the homogeneity and the moisture content of the prepared silica gel by determining the moisture content gravimetrically in three different samples. The moisture content should not vary more than 0.1%.

Evenly add approximately 5 g of prepared silica gel to the column and top with approximately 3 g of sodium sulfate (dried for 4 hours at about 180°C). After rinsing with 30 mL of hexane, the column is ready for use.

NOTE: Hexane may be used as a moistening agent during the filling process.

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2. After the 2 M HCl wash, add a 1.0 mL aliquot of the extract to the head of a silica gel cleanup column. Rinse the ampule/vial onto the column using hexane.
3. Slowly add 1mL of hexane (containing ~0.1 mg/L naphthalene) onto the column. After penetration of the solvent, add 20 mL hexane (containing ~0.1 mg/L naphthalene) solution and collect the eluent in a container suitable for solvent reduction.
4. Reduce the volume of the organic phase to 1.0 mL using a suitable apparatus. Avoid reducing the extract to dryness.

NOTE: Evaporation step should be performed slowly; if done too quickly there will be a loss of target compounds (esp. methyltins).

V. Analysis

See the ORTEP Method “Gas Chromatographic Analysis of Derivatized Organotin Chlorides” for details of the GC-MS, GC-FPD, or GC/AED analysis.

VI. Calculations

See the ORTEP Method “Gas Chromatographic Analysis of Derivatized Organotin Chlorides” for details of analytical calculations and data reporting.

VII. References

de Wolf, J.M. and R. Schilt. 2004. Validation of analysis of organotin chlorides MMTC, MBTC, DBTC, MOTC, DOTC and TOTC, and tetraalkyltins TTOT and TTBT in RM3 rodent diet. TNO Report V4921. Study conducted by TNO Nutrition and Food Research, Analytical Sciences for the ORTEP Stabilizer Task Force. Ziest, The Netherlands.

DIN Deutsches Institut für Normung e.V. 2001. DIN 38407 – 13:2001-03 German Standard Methods for the Examination of Water, Waste Water and Sludge – Jointly Determinable Substances (Group F) – Part 13: Determination of selected organotin compounds by gas chromatography (F13). Beuth Verlag, 10772 Berlin, Germany.

ORTEP. 2006. ORTEP Stabilizer Task Force (STF) Analytical Method - Gas Chromatographic Analysis of Derivatized Alkyltin Chlorides. Revision 3.0. January 2006.