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Title: Determination and Confirmation of Chloramphenicol		
Revision: 04	Replaces: CLG-CAM.03	Effective: 10/09/2009

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A. INTRODUCTION

1. Theory

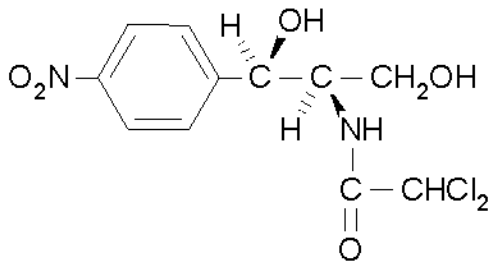
Chloramphenicol (CAM) has a broad spectrum of activity against gram-positive and gram-negative bacteria and rickettsia. It is banned in the U.S. for use in animals used as food.

Meta-chloramphenicol is added to the sample as a recovery index. The sample is then incubated with β-glucuronidase to convert any chloramphenicol monoglucuronide to free chloramphenicol. CAM is extracted from muscle with ethyl acetate and the ethyl acetate is concentrated to about 1 mL. A 4% sodium chloride solution is added and the remaining ethyl acetate is purged with nitrogen. The salt solution is applied to the top of a C_{18} SPE column, the cartridge is washed with methanol: water (20:80) and the CAM is eluted with acetonitrile. The eluate is evaporated to dryness and silanized. CAM is quantitated by GC/ECD using a DB-1 capillary column. Confirmation is accomplished by GC/MS, using an OV-1 capillary column and negative ion chemical ionization.

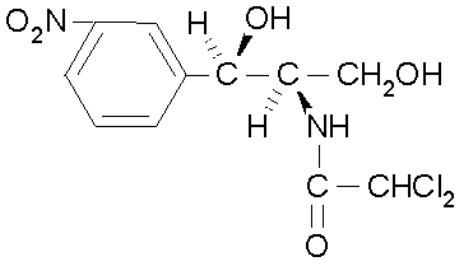
2. Applicability

This method is applicable for bovine muscle at concentrations ≥ 0.25 ppb, and turkey and catfish muscle at concentrations ≥ 0.3 ppb.

3. Structure



Chloramphenicol



Metachloramphenicol

B. EQUIPMENT

Note: Equivalent apparatus or instrumentation may be substituted for any of the following.

1. Apparatus
   a. Vacuum manifold device - for aid in washing and elution of C18 cartridges. Optional, but allows for multiple C18 elutions. J.T. Baker or Analytichem.
   b. N-Evap - Organomation Associates.
   c. Pipettes - disposable, glass serological (10 mL) and Eppendorf (50-200 µL).
   d. Test tube racks.
   e. 50 mL polypropylene conical tubes - Falcon BlueMax.
   f. Microliter syringes - 10 µL, for injection. Hamilton #701 and #1 701.
   g. Centrifuge - Damon IEC Division Model #PR-7000 with IEC #253 rotor, cup size IEC catalog #320.
   h. Vortex mixer - Labline Supermixer Model #1290.
   i. Homogenizer - Ultra Turrax, Tekmar Model SDT, with microshaft.
   j. Incubator - low temperature, Precision Scientific Freas Model 825.
   k. Heating module - Reacti-therm, Pierce Model #18780.
   l. Conical 1 mL autosampler vials - available from Chemical Research Supplies, combo pack with polyethylene P8-6.
   m. Culture tubes - 5 mL borosilicate, dimensions 13 x 10 mm, Corning #99445.
   n. Pasteur pipettes - Kimble #72050.
   o. Baker 10 SPE C18 (octadecyl) columns - 3 mL capacity, Catalog No. 7020-3.
   p. Borosilicate glass disposable centrifuge tube - 15 mL screwthread, Kimble #73785-15 and phenolic cap (PTFE faced, rubber liner), Kimble #73802-15415.
   q. Shaker - Eberbach, variable speed.
   r. Robot Coupe processor - Robot Coupe U.S.A., Inc.
2. Instrumentation
   a. Gas chromatograph - Hewlett-Packard 6890 with capillary inlet (splitless injection) fitted with electron capture Ni-63 detector.
   b. Gas chromatographic column - DB-1, 30 meter length, 0.254 mm i.d., having a film thickness of 0.25 µm, J&W Scientific.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents and solutions may be substituted for any of the following.

1. Reagents

   a. Methanol - High-purity solvent product 230-4, Burdick and Jackson (B&J).

   b. Ethyl acetate - Omni Solv product EX0241 -1.

   c. Hexane - UV, B&J high-purity solvent product 216.

   d. High-purity water - 18 megaohm/cm specific resistance.

   e. Acetonitrile - UV, B&J high-purity solvent product 015.

   f. Cyclohexane - pesticide grade, Fisher C-553.

   g. Potassium Phosphate, Monobasic - ACS grade, Sigma, P-0662.

   h. Sodium Phosphate, Dibasic - Sigma, S-5136.

   i. β-glucuronidase - Sigma, G-7396.

   j. Sylon HTP - Supelco, 3-3038.

   k. Sodium Chloride - ACS grade, Mallinckrodt, 7581.

2. Solutions

   a. Type IX-a β-glucuronidase:

      Dilute with buffer (refer to item b below) to a concentration of 4,000 units/mL. Use the units/gram listed on the bottle to calculate the amount of dry reagent needed. Prepare fresh daily. Store dry β-glucuronidase below 0 ºC.

   b. Buffer solution:

      Mix approximately 41 g of KH_2PO_4 and 43 g of Na_2HPO_4 (ACS reagent grade) in 3 L of water aqueous, pH 6.8 ± 0.1. Adjust pH to 6.8 with the appropriate dry reagent.

   c. 4 % Sodium chloride:

      Prepare 4% aqueous solution with distilled water, by mixing 20.8 g of sodium chloride with 500 mL of water. Store at room temperature.

   d. Cyclohexane/hexane (60:40):

      Mix 60 mL cyclohexane and 40 mL hexane in a graduated cylinder.

   e. Methanol/Water (20:80):

      Mix 200 mL methanol and 800 mL deionized water in a 1 L volumetric flask.

D. STANDARDS

1. Source

   a. Chloramphenicol
   Sigma Chemical Co.

   b. Metachloramphenicol (Internal Standard)

   Sigma Chemical Co.

2. Preparation of Standard Solutions

   a. Stock Solution (500 µg/mL):

   Prepare individual 500 µg/mL stock solutions of chloramphenicol and metachloramphenicol by transferring 50 mg standard to a 100 mL volumetric flask and diluting to volume with methanol.

   b. Intermediate Solution (50 µg/mL):

   Prepare individual intermediate solutions at 50 µg/mL by transferring 10 mL of stock into a 100 mL volumetric flask and diluting to volume with methanol.

   c. Working Solutions (100 ng/mL):

   Prepare individual working solutions at 100 ng/mL by transferring 200 µL of the intermediate solution into a 100 mL volumetric flask and diluting to volume with methanol.

3. Storage conditions

   All standards should be stored in amber glassware in a freezer, unless specified otherwise by the manufacturer.

4. Stability

   a. Stock: one year

   b. Intermediate standards: 6 months

   c. Working standards: 3 months

   Note: This is applicable to both internal and chloramphenicol standards when stored under the above conditions.

E. SAMPLE PREPARATION

Prepare muscle tissue as follows:

1. Cut lean tissue from different parts of muscle sample. Avoid the fat and connective tissue as much as possible.

2. Cut just enough of the muscle to make the sample (approximately ¾ to 1 pound).

3. Cut the muscle tissue into small ½ to 1 inch cubes before processing. Cut the muscle tissue into cubes smaller than ½ to 1 inch if the sample tissue is tough.

4. Process the muscle tissue using Robot Coupe or grinder until the sample is homogeneous.

F. ANALYTICAL PROCEDURE

1. Sample Extraction
   a. Weigh 10 g previously ground muscle tissue into a 50 mL centrifuge tube.
   b. To each sample add 100 µL metachloramphenicol internal standard (100 ng/mL in methanol, 1 ppb).
   c. Prepare 1 blank muscle and 3 fortified blank muscle samples to be analyzed with each sample set. Add internal standard to the control tissues. Fortify the recovery curve samples at 0.5 ppb (50 µL of working standard), 1.0 ppb (100 µL of working standard), and 2.0 ppb (200 µL of working standard). Data generated from fortified samples will be used for calculations.

      Note: To quantitate positives below 0.5 ppb prepare a fortified curve at 0.25 ppb, 0.5 ppb, and 1.0 ppb.
   d. Add 15 mL phosphate buffer (pH 6.8 ± 0.1) and 200 µL ß-glucuronidase (800 units) solution to all blanks, fortified controls, and sample tubes.
   e. Blend in a tissuemizer for 30-60 sec at room temperature or shake on a mechanical shaker at high speed for at least 5 minutes.
   f. Incubate all tubes at least 90 min at 37 ± 2 °C. After incubation, samples may be left in refrigerator overnight.
   g. Bring tubes to room temperature.
   h. Add 15 mL ethyl acetate to each tube.
   i. Mix tubes on vortex mixer for 30 sec or shake on a mechanical shaker for one minute to extract chloramphenicol.
   j. Centrifuge at approximately 2,000 rpm for at least 2 min to separate phases.
   k. Transfer ethyl acetate (upper phase) with a disposable pipette to a clean 15 mL tube. Evaporate extract to approximately 1 mL at 65 ± 5 °C.

l. Repeat extraction of sample (steps h-k) and combine extracts.

m. Reduce ethyl acetate volume to 1 mL on an N-Evap, or equivalent, under a gentle stream of nitrogen, using a sand bath or water bath temperature of approximately 65 ± 5 °C.

n. Add 4 mL aqueous 4% NaCl solution to all tubes and vortex for approximately 5 - 10 sec.

o. Continue evaporation of ethyl acetate on N-Evap until ethyl acetate layer is absent, leaving an oily residue over the NaCl solution.

p. Add 5 mL hexane to the 4 mL 4% aqueous NaCl layer. Vortex for 10 sec. Centrifuge at 1000 rpm for 1 min. Remove top layer and discard.

q. Repeat step p.

Note: Steps r through u should be performed immediately, one after the other. Do not allow the sorbent to dry.

r. Preconditon a C18 column for each sample, blank, and fortified control by passing 10 mL methanol, and 10 mL distilled water sequentially through the column. Discard all washes.

s. Transfer the entire aqueous extract onto the C18 column. Discard the eluate.

t. Rinse the sample tube by vortexing twice with 1 mL distilled water and adding the rinses onto the C18 column. Discard eluate.

u. Wash each C18 column with 1 mL water followed by 2 mL methanol-water (20:80). Allow the last wash to elute completely through the column. Discard washes.

v. Elute the chloramphenicol from the C18 column with acetonitrile, 3 mL, collecting the eluate in a clean 5 mL culture tube.

Note: Samples may be stored in a freezer at this point.

w. Evaporate the acetonitrile eluate to dryness using a sand bath or water bath at a temperature of 65 ± 5 °C and a gentle stream of nitrogen.

CAUTION: Avoid moisture from this point forward.

x. To the dried residue, add 200 µL Sylon HTP.

y. Stopper and vortex 5 sec. React at 65 ± 5 °C in a sand bath or water bath for 15 min.

z. Evaporate excess reagents on heating module or water bath at 65 ± 5 °C with gentle stream of nitrogen to approximately 10 µL.

CAUTION: Excessive drying time at this step may result in loss of analyte.

aa. Reconstitute residue in 200 µL cyclohexane/hexane (60:40). Vortex 5 sec.

bb. Inject suitable microliter volume of derivatized material into GC for quantitative determination, or into GC/MS for confirmation.

2. Instrumental Settings and Conditions

The following conditions are for the 6890 Hewlett-Packard GC as described in section B.2 and should be considered an example only. The analyst should optimize these parameters for the instrument being used.

a.	Carrier gas:	Helium, linear velocity 15 cm/sec
b.	Make-up gas:	Argon/methane, 95/5, flow rate 50 mL/min
c.	Initial column temperature:	100 °C, hold for 1 min
d.	Temperature programming:	Program at 20 °C/min to 290 °C; hold for 15 min or until the Meta isomer and chloramphenicol have eluted. Then program at 20 °C/min to 310 °C; hold for 2 min to make sure all the sample has eluted.
e.	Injector temperature:	260 °C
f.	Detector temperature:	330 °C
g.	Sensitivity setting:	2/8 attenuation
h.	Expected retention time:	Chloramphenicol 11 to 16 min metachloramphenicol 11 to 16 min
i.	Expected response:	50 % full-scale deflection for 0.20 ng chloramphenicol

3. Interferences

No known interferences.

4. Sample Chromatograms

See Part I (Determinative method), Section K, for chromatograms.

G. CALCULATIONS

1. Procedure

a. Metachloramphenicol is used as an internal standard for calculating chloramphenicol concentration. Proceed in the following manner to calculate linear regression calibration curves and chloramphenicol concentration. By an acceptable means, measure the peak height or peak area for each component in the fortified samples that have been processed through the procedure. Calculate the peak height or peak area ratios for chloramphenicol by dividing its peak height or peak area by that of the metachloramphenicol peak height or peak area.

b. Using the ratios and associated ppb values, calculate a linear regression calibration curve by least squares computation.

$y = mx + b$, where

m = slope

b = y intercept

$$y = \frac{\text{chloramphenicol peak height or area}}{\text{metachloramphenicol peak height or area}}$$

x = chloramphenicol concentration in ppb

This calibration curve is then used to calculate values for additional samples from the sample set.

H. HAZARD ANALYSIS

1. Required Protective Equipment - Safety glasses, polyvinyl or latex gloves, and lab coat.

2. Hazards

CAUTION: Do not swallow, inhale, or absorb through skin any chemical, as complete toxicological properties for most chemicals are unknown.

Reagents	*Hazards*	*Recommended Safe Procedures*
Enzyme: β-glucuronidase, type IX-a	May be harmful by inhalation, ingestion/skin absorption. May cause irritation/allergic reactions in certain sensitive individuals.	The analysis should be done under an efficient fume hood.
Ethyl acetate, Hexane, Methanol, Cyclohexane	Flammable. Vapors are corrosive to the skin, eyes, and respiratory system.	Avoid contact or prolonged exposure to vapors. Work in a fume hood. Keep away from flame or heat.
Analyte: Chloramphenicol Metachloramphenicol	Chloramphenicol has a wide range of possible adverse effects ranging from dermatitis, embryo and fetal death, to death of adults. Chloramphenicol has been associated with inducing aplastic anemia. Those who survive aplastic anemia have a high incidence of developing acute leukemia. It is considered a carcinogen. The Meta isomer is assumed to have similar toxicity	Because the unique toxicity to humans has been well documented, the utmost care in handling chloramphenicol is recommended. See the material safety data sheet for information on how to handle any spills.

3. Disposal Procedures

Procedure Step	Hazard	Recommended Safe Procedures
Organic solvents and chloramphenicol solutions.	See above	Collect waste in tightly sealed container and store away from non-compatibles in a cool, well ventilated, flammable liquid storage area/cabinet for disposal in accordance with local, state, and Federal regulations.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

Analyte	Analytical Range (ppb)	Acceptable Recovery %	Acceptable Repeatability (CV)
Chloramphenicol	0.25 - .49 0.5 - 2.0	77 -131 85 -115	< 20 at 0.25 ppb < 20 at 1.0 ppb

Standard curve correlation coefficient ≥ 0.9945.

2. Critical Control Points and Specifications

	Record	***Acceptable Control***
a.	(ß-glucuronidase) solution	Prepare fresh daily.
b.	Incubation time	Minimum incubation time is 90 minutes.
c.	Incubation temperature	37 ± 2 °C
e.	Sample size	10
f.	Working standard solution volume	Depends on fortification level.
g.	Sylon HTP volume	200 µL ± 20 µL
h.	Reaction time	15 min ± 1 min
i.	Reaction temperature	65 ± 5 °C
k.	Evaporation of excess Sylon HTP	See section F.1. z.

3. Readiness To Perform

   a. Familiarization

      i. Phase I: Standards- 5 levels, 3 replicates each, on 3 separate days:

         (a) Blank (0 ng/μL)

         (b) 0.25 ppb (0.025 ng/μL) for beef muscle or 0.3 ppb for turkey and catfish muscle.

         (c) 0.5 ppb (0.05 ng/μL)

         (d) 1.0 ppb (0.10 ng/μL)

         (e) 2.0 ppb (0.20 ng/μL)

         NOTE: ppb calculated based on 10g sample.

      ii. Phase II: Fortified samples- 5 levels, 3 replicates each. Same levels as above - over a minimum of 3 days, 15 total samples. The number of replicates is at the discretion of the supervisor.

         NOTE: Phase I and Phase II may be performed concurrently.

      iii. Phase III: Check samples for analyst accreditation.

         (a) Eight samples fortified between 0.25 ppb (for beef muscle) or 0.3 ppb (for turkey or catfish muscle) and 2.0 ppb.

         (b) Samples submitted by the supervisor.

         (c) Report analytical findings to the Quality Assurance Manager (QAM).

         (d) Letter from QAM is required to commence official analysis.

   b. Acceptability criteria.

      Refer to Section I. 1 above.

4. Intralaboratory Check Samples

   a. System, minimum contents.

      i. Frequency:

         (a) One sample weekly per analyst as samples analyzed.

         (b) Random replicates may be chosen by the supervisor or his/her designee.

      ii. Records are to be maintained.

   b. Acceptability criteria.

      If unacceptable values are obtained, then:

i. Stop all official analyses by that analyst.

ii. Take corrective action.

5. Sample Acceptability and Stability

   a. Matrix: Bovine, turkey, and catfish muscle

   b. Sample receipt size: 1 pound

   c. Condition upon receipt : Cold

   d. Sample storage:

      i. Time: 6 months

      ii. Condition: Frozen

6. Sample Set

   a. Each sample set should include:

      i. Tissue blank

      ii. Fortified blanks at 0.25 ppb (if needed), 0.5 ppb, 1.0 ppb, and 2.0 ppb

      iii. Samples

7. Sensitivity

   a. Lowest detectable level (LDL): To be determined

   b. Minimum proficiency level (MPL): 0.25 ppb for beef muscle and 0.3 ppb for turkey and catfish muscle.

J. WORKSHEET

The worksheet on the following page can be removed for photocopying.

DATASHEET: CHLORAMPHENICOL

		Critical Control Points	
		Sample Size	10.0 g
Analyst		Incubation Time	90 minutes minumum
Analyst Code		Incubation Temp	37 ± 2 °C
Matrix		Chloramphenicol *Std ILN*	
Date Started		Meta *Std ILN*	
Date Completed		Sylon HTP Volume	200 ± 20 uL
Rec ppb		Reaction Temp.	65 ± 5 °C
Check ppb		Reaction Time	15 ± 1 min

Standard Curve

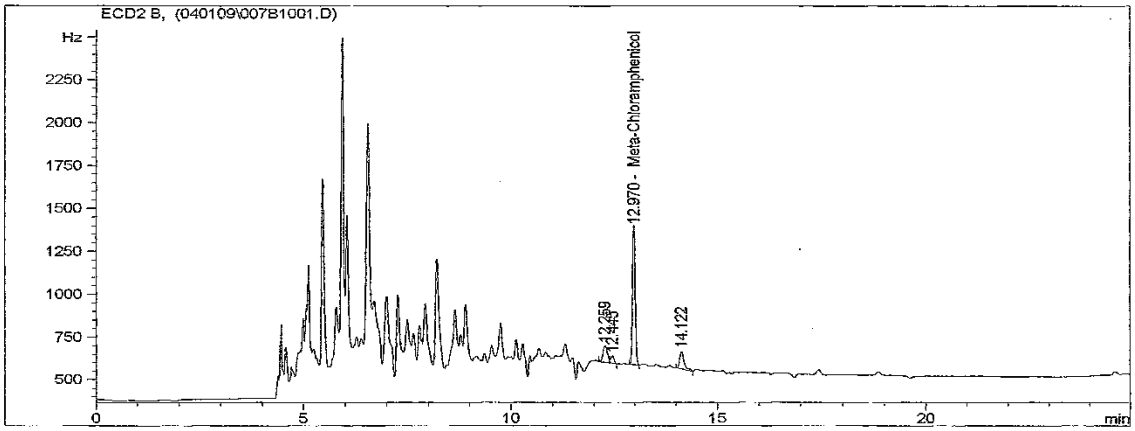
Fortification	Retention Time (min)		Peak Ht/Area counts		CAP/Meta	Linear Regression
(ppb)	meta	CAP	meta	CAP	Ratio	Results
0.0						m =
0.5						b =
1.0						r =
2.0						s =

Sample Analysis

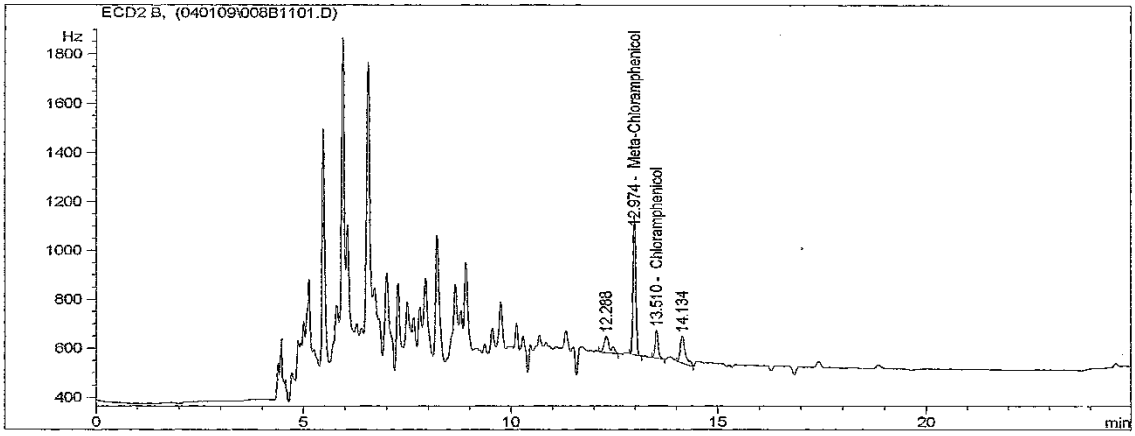
Sample ID	QA Type	Ret. Time (min)		Peak Ht./ Area Counts		CAP/Meta		
		meta	CAP	meta	CAP	Ratio	ppb	% Rec.
QC								
%Steadiness		#DIV/0!	#DIV/0!			#VALUE!		

K. CHROMATOGRAMS AND SPECTRA

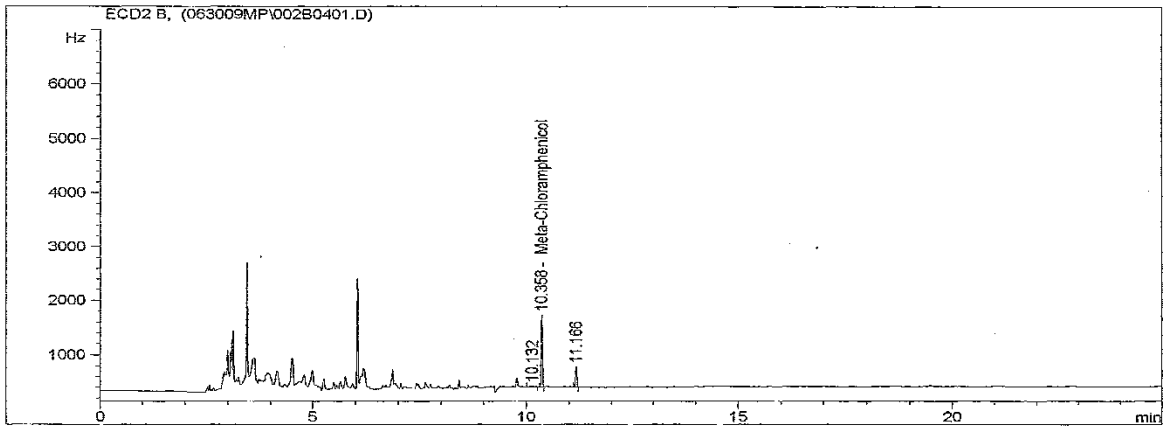
1. Blank bovine muscle



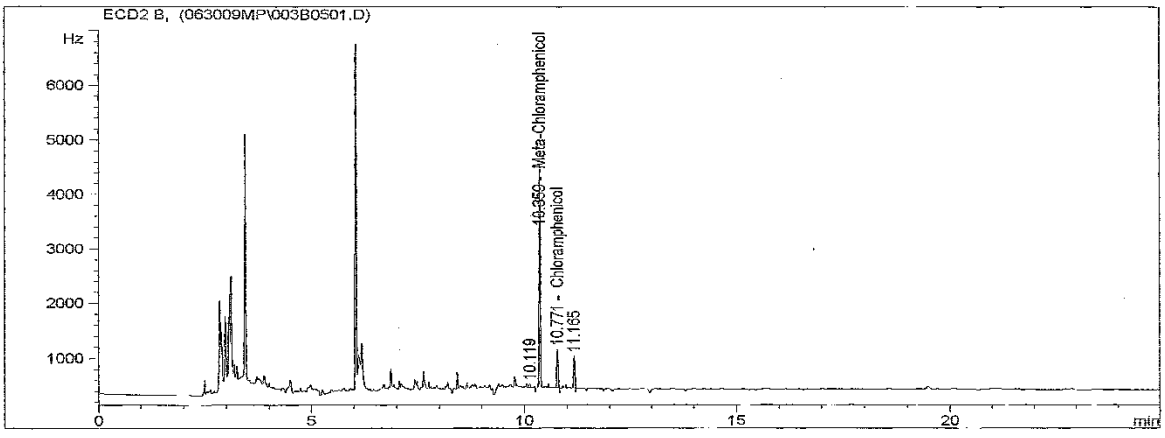
2. Bovine muscle fortified with 0.25 ppb chloramphenicol



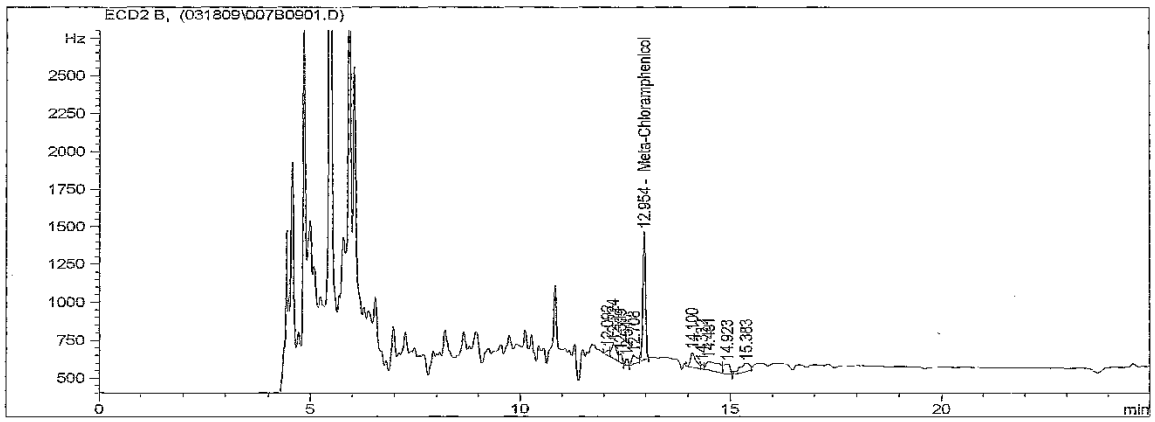
3. Blank turkey muscle



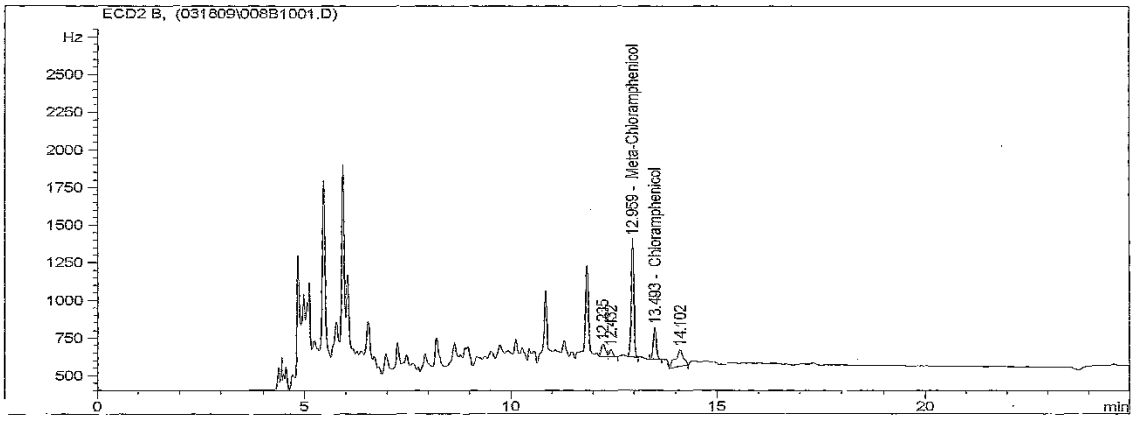
4. Turkey muscle fortified with 0.3 ppb chloramphenicol



5. Blank catfish muscle



6. Catfish muscle fortified with 0.3 ppb chloramphenicol



II. Confirmatory Method

A. INTRODUCTION

Refer to Determinative Method, section A.

B. EQUIPMENT

1. Apparatus

   Refer to Determinative Method, section B.1

2. Instrumentation

   GC/MS - HP 6890/5973N Mass Selective Detector, quadrupole with capillary inlet, splitless injection fitted crosslink methyl silicone column, film thickness 0.33 um, length, 25 meters, diameter 0.20 mm, column part number 19091S-602.

C. REAGENTS AND SOLUTIONS

1. Reagents

   a. Perfluorotributylamine (PFTBA)

   b. Refer to Determinative Method, section C.

D. STANDARDS

1. Source

   a. See Determinative Method, section D.

   b. The mass spectrometer operator will use the same sample set that is used in the Determinative Method for confirmation. Only the 1.0 ppb standard is usually analyzed.

E. SAMPLE PREPARATION

A suitable microliter volume of derivatized material obtained in the determinative procedure (see Determinative Method, section E) is injected into the GC/MS for confirmation.

F. ANALYTICAL PROCEDURE

1. Data Acquisition

   Note: The following conditions are for the instrument described in section B.2 and are

given as an example only. The analyst should optimize the parameters for the instrument being used.

a. GC conditions and parameters

i.	Column:	Crosslinked Methyl Siloxane, Capillary, 25 meters
ii.	Injector:	230 °C
iii.	Temperature programming:	150 °C with no initial hold, program to 300 °C at 20 °C/min. Final hold 10 min.
iv.	Transfer line temperature:	300 °C
v.	Helium flow rate:	29 cm/sec, splitless injection

b. Mass spectrometer conditions

i.	Detection:	Negative ion chemical ionization (NICI)
ii.	Ionization gas:	Methane
iii.	Source temperature:	260 °C
iv.	Mode of operation:	SIM-NICI
v.	Dwell time:	100 millisec
vi.	Calibration standard:	PFTBA: Tune acceptance is based on manufacturer's instructions.

2. Required Samples for GC/MS Analysis

Set up instrument as described in section 1 above to monitor chloramphenicol and the internal standard metachloramphenicol. Inject 2 to 5 µL of the external standard and analyze results to verify that the system is functioning properly. If not, make any necessary adjustments in operating parameters or standard concentration, then re-inject to verify performance. Inject 2 to 5 µL each of the confirmation sample, the recovery, and the tissue blank. Confirmatory analysis is required in all samples found positive by the determinative method.

3. Criteria for Confirmation

a. Retention time specification. Compare the retention time of the sample with the retention time of the fortified tissue. The times should match ± 2.0%.

The following is an example only as other retention times are acceptable.

Compound	*Expected Retention Time (min)*
Metachloramphenicol	7.7 ± 0.15
Chloramphenicol	7.9 ± 0.16

b. Ions 468, 466, 322, and 304 must be present. In samples with high levels of chloramphenicol, ions m/z 358 and 360 can also be monitored.

c. Compute at least the following three ratios for the standard, fortified control, and samples: 466/468, 322/466, and 304/466.

For successful confirmation between levels 0.25 ppb and 2.0 ppb, the 466/468 and either the 322/466 or the 304/466 ion for the sample must agree within ± 20% relative of the ratio for fortified tissue.

In samples with high levels of chloramphenicol, ion ratios for 358/466 and 358/360 may also be used to confirm.

The ion ratios of the fortified standard must match the ion ratios of the sample by ± 20 % relative. The following are expected ion ratios for the conditions cited.

466/468 = 1.43

322/466 = 0.49

304/466 = 0.57

Note: The ion ratios are dependent upon the source temperature.

Refer to the following page for postulated structured of confirming ions.

4. Interferences

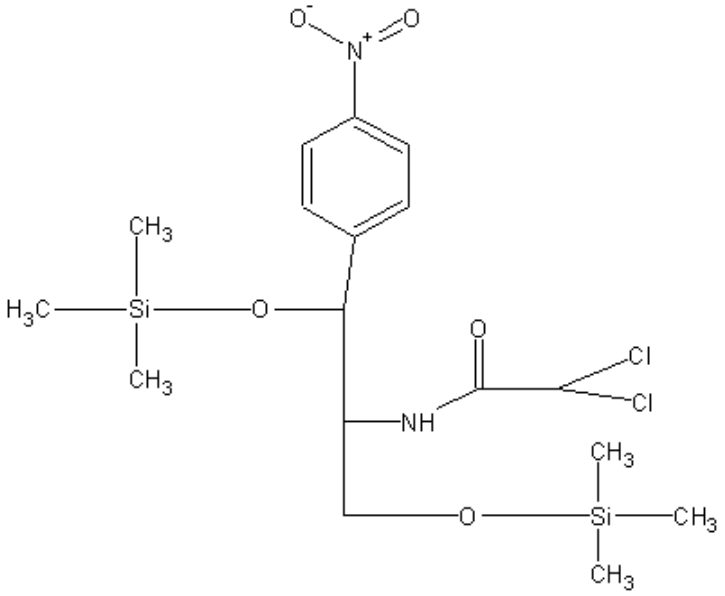
No known interferences.

5. Postulated Structure of some confirming Ions.

MASS

468 AND 466

Due to Cl isotopes



6. Sample Chromatograms

See Part II (Confirmatory method), section K, for chromatograms.

G. CALCULATIONS (Not Applicable)

H. HAZARD ANALYSIS

Refer to Determinative Method, section H.

I. QUALITY ASSURANCE PLAN

1. Performance Standard
   a. No false positives at 0 ppb.
   b. No false negatives at 0.25 ppb.
2. Critical Control Points and Specifications

See Determinative Method, section I.2.

3. Readiness To Perform
   a. Familiarization
      i. Phase I: Standards- At least 3 replicate injections of 1.0 ppb standards.
      ii. Phase II: Fortified samples- Minimum of 3 samples, with at least 2 positives between 0.25 ppb and 2 ppb.

      Note: Phase I and Phase II may be performed concurrently.
      iii. Phase III: Check samples for analyst accreditation.
         (a) 6 samples fortified between 0.25 ppb and 2.0 ppb submitted by the supervisor.
         (b) Report analytical findings to the Quality Assurance Manager (QAM).

   b. Acceptability criteria.

      Refer to Section II. I.1 above.

4. Intralaboratory Check Samples
   a. Random replicates may be chosen by the supervisor or his/her designee.
   b. Frequency: One sample weekly per analyst as samples analyzed.
   c. Acceptability criteria.

      If unacceptable values are obtained, then:
      i. Stop all official analyses by that analyst.
      ii. Take corrective action.

5. Sample Acceptability and Stability
   a. Sample storage: Samples are stable for six months if stored in a freezer.
   b. Condition of extract upon receipt: Cold.

6. Sample Set
   a. Standard
   b. Tissue Blank
   c. Tissue fortified at level of interest.
   d. Samples

7. Sensitivity
   a. Lowest detectable level (LDL): Not Applicable
   b. Lowest reliable confirmation (LRC): Validated to 0.25 ppb for beef muscle and

0.3 ppb for turkey and catfish muscle.

J. WORKSHEET

The worksheet can be removed for photocopying.

CHLORAMPHENICOL CONFIRMATION DATASHEET

GC/MS Analyst
Analyst Code
Date Started
Date Finished
Sample Wt. 10+/-0.1g
Injection Vol

GC Conditions
Carrier Gas Helium
Injector Temp. 230 C
Transfer Line Temp 300 C

MS Conditions
Detection NCI
Ionization Gas Methane
Source Temp 260 C
Quadrupole Temp 150 C
Mode of Operation SIM

			Ion Abundance						Ion Ratios				
Std & Rec.	Ret Time	Weight	466	468	322	304	358	360	466/468	322/466	304/466	358/466	358/360
Standard													
Recovery													

			Ion Abundance						Ion Ratios					Std Ret	Rec. Ret	
Sample	Ret Time	Weight	466	468	322	304	358	360	466/468	322/466	304/466	358/466	358/360	Time Corr	Time Corr	Confirmed?

Range for Standard

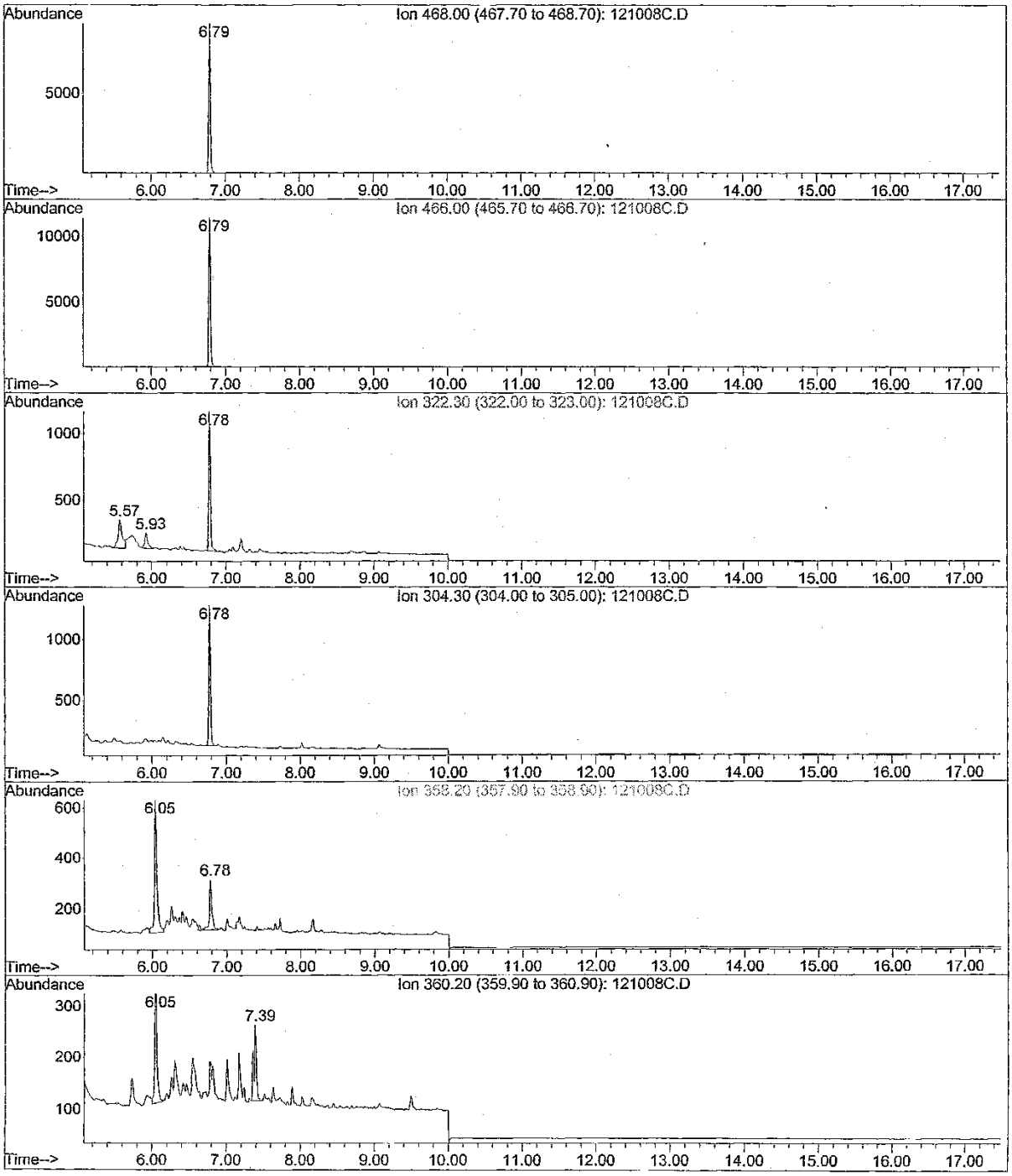
Ion	-20%	+20%
466/468		
322/466		
304/466		

Range for Recovery

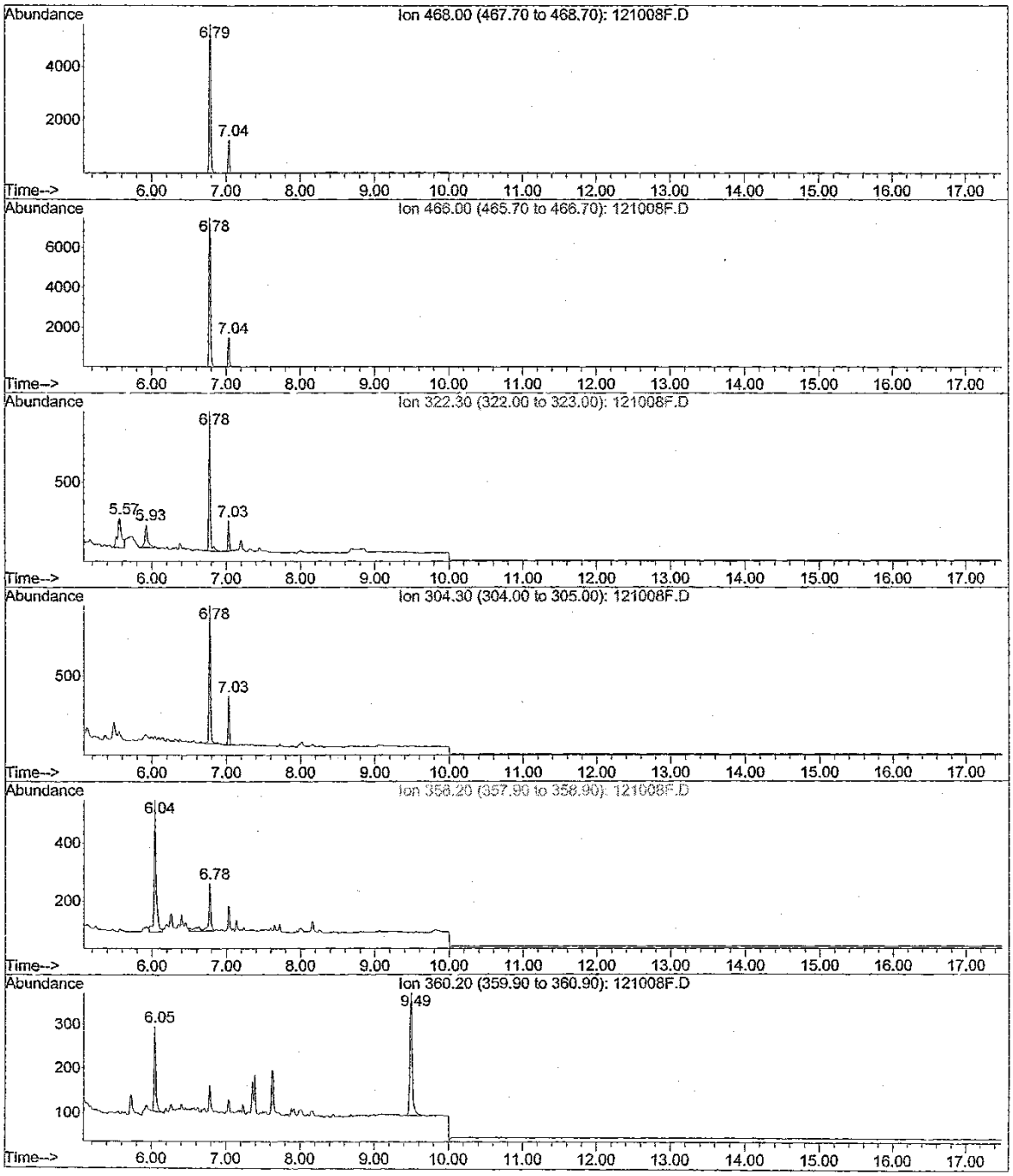
Ion	-20%	+20%
466/468		
322/466		
304/466		

K. CHROMATOGRAMS AND SPECTRA

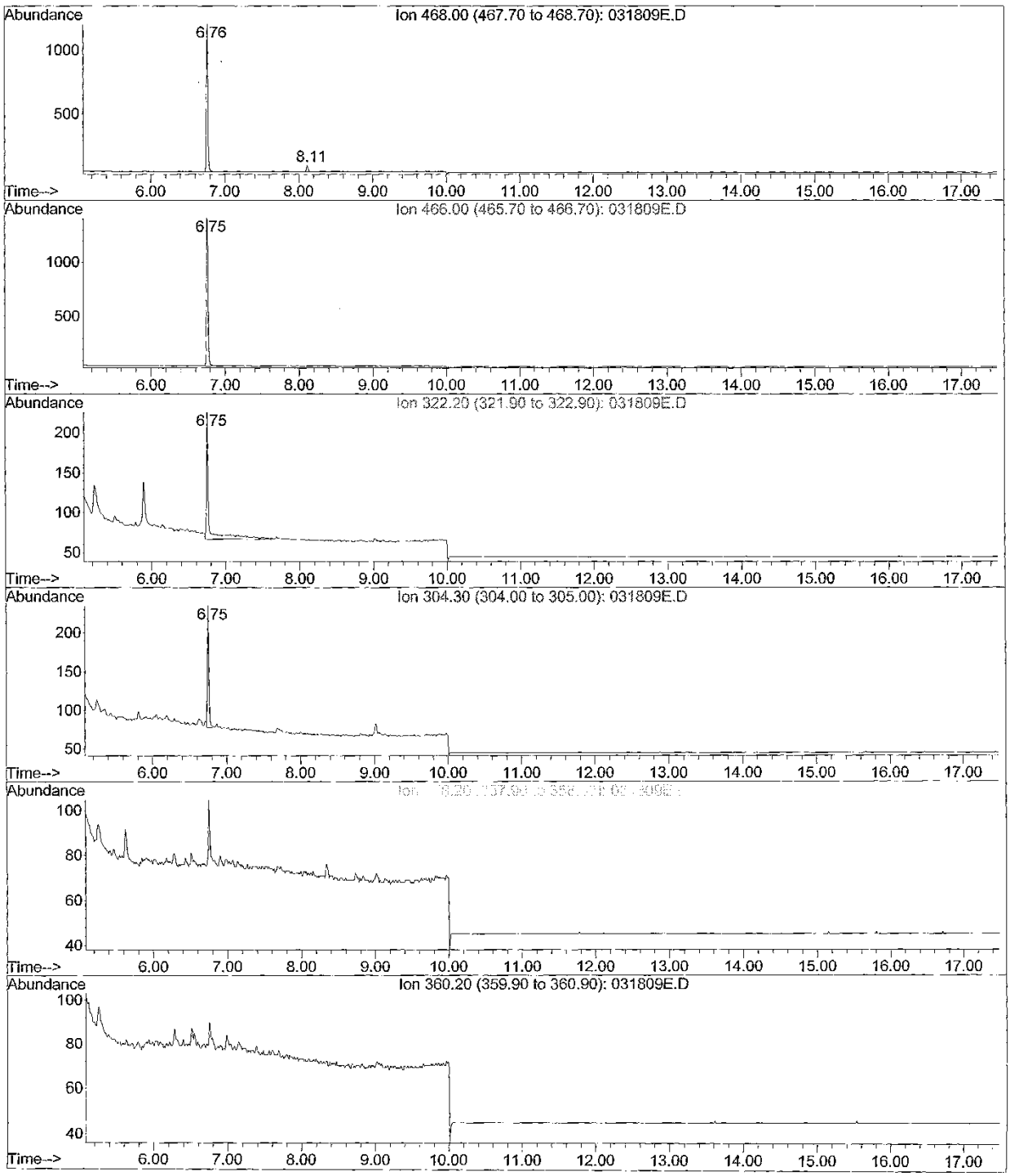
1. Blank turkey muscle



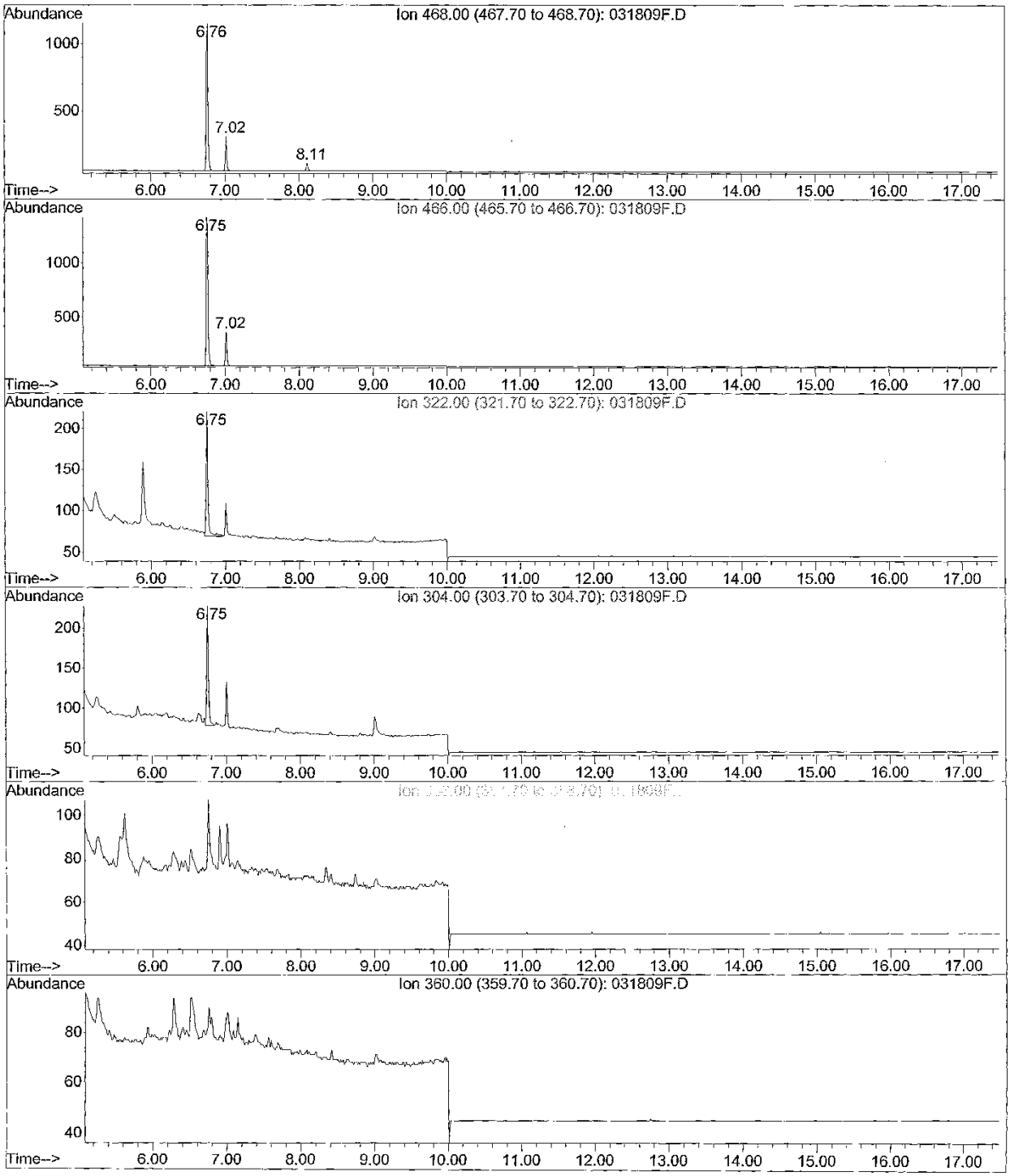
2. Turkey muscle fortified with 0.3 ppb chloramphenicol



3. Blank catfish muscle



4. Catfish muscle fortified with 0.3 ppb chloramphenicol



L. APPROVALS AND AUTHORITIES

Approvals on file.

Issuing Authority: Director, Laboratory Quality Assurance Division.