

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02	Page 1 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

Contents

A.	INTRODUCTION	2
B.	EQUIPMENT	4
C.	REAGENTS AND SOLUTIONS	5
D.	STANDARDS	5
E.	SAMPLE PREPARATION	7
F.	ANALYTICAL PROCEDURE.....	7
G.	IDENTIFICATION	11
H.	SAFETY INFORMATION AND PRECAUTIONS.....	12
I.	QUALITY ASSURANCE PLAN	13
J.	WORKSHEET	15
K.	APPENDIX	18
L.	APPROVALS AND AUTHORITIES	26

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02	Page 2 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

A. INTRODUCTION

1. Theory

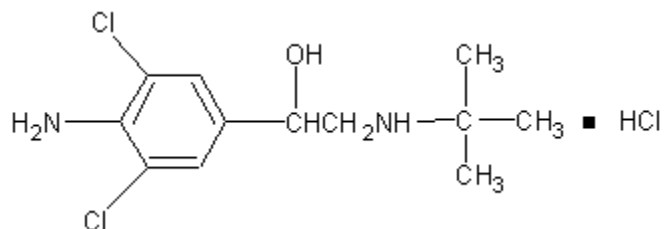
Free residues of clenbuterol, salbutamol, cimaterol, zilpaterol, and ractopamine are extracted from retinal, liver, or muscle tissues with a mixture of acetonitrile, isopropanol, sodium chloride, sodium sulfate, and magnesium sulfate. This extract is evaporated, reconstituted in water, filtered, and analyzed by LC/MS/MS.

2. Applicability

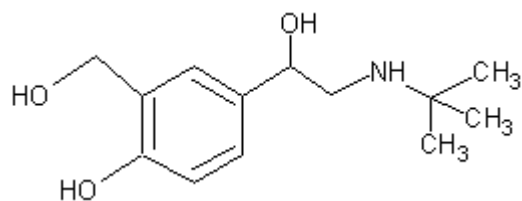
This method is applicable to identification of β -agonists in bovine retinal tissue (except for zilpaterol); bovine, porcine, ovine, and caprine liver; and bovine and porcine muscle at ≥ 3 ppb for clenbuterol, salbutamol, and cimaterol; ≥ 6 ppb for zilpaterol; and ≥ 21 ppb for ractopamine.

3. Structure

Note: For proposed fragmentation paths, see Appendix, K.3.



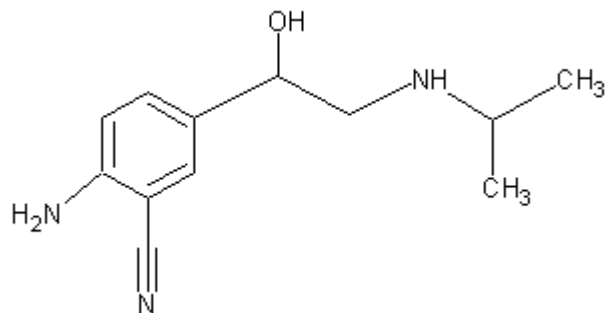
Clenbuterol Hydrochloride



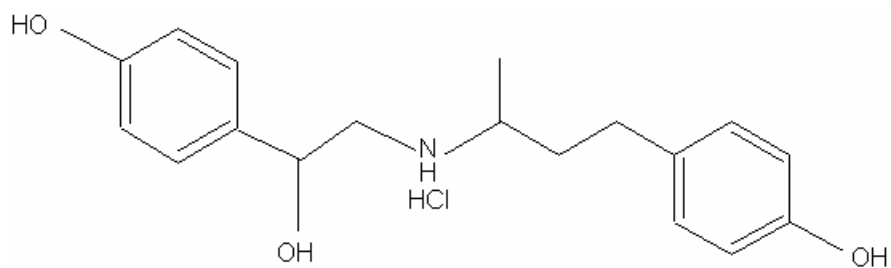
Salbutamol

United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science

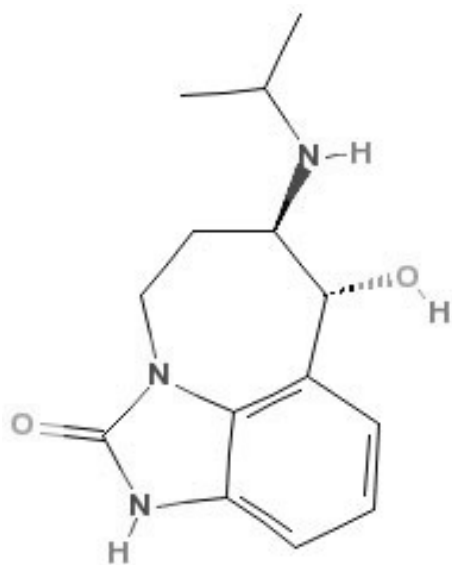
CLG-AGON1.02	Page 3 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007



Cimaterol



Ractopamine HCl



Zilpaterol

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02	Page 4 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

B. EQUIPMENT

Note: Equivalent apparatus may be substituted for the following:

1. Apparatus

- a. Petri Dishes – disposable polystyrene, Optilux 100 x 20 mm, Cat. No. 1005, Becton Dickinson.
- b. Scalpels – Fisherbrand single-use scalpel, Cat. No. 08-927-5D, Fisher, or Razor Blades – Single edge safety razor, American Safety Razor Co.
- c. Cut Resistant Glove – Cat. No. FSIS-50, FSIS Beltsville Service Center, or Ansell Polar Bear Glove, Cat. No. 19-058-952, Fisher.
- d. Waring commercial blender Model 51BL31.
- e. Tissuemizer – Polytron, Brinkmann Instruments Ltd.
- f. Top loading balance – 0.01 g sensitivity, PJ3600 DeltaRange, Mettler.
- g. Centrifuge tubes – 50 mL round bottom polyallomer with sealing caps, Cat. No. 3138-0050, Nalgene.
- h. Centrifuge tubes – 50 mL conical, disposable, polypropylene, with caps, Cat. No. 352098, Becton Dickinson.
- i. Micropipetters – Adjustable, 10 – 5000 μ L, Eppendorf.
- j. Glass rods – 10 mm diameter by 25 cm length, fired and rounded at both ends.
- k. Vortex mixer – variable speed, Cat. No. S8223-1, American Scientific Products.
- l. Shaker – Horizontal flatbed, two speed, Cat. No. 511105, Eberbach.
- m. Centrifuge – International Equipment Company B-22M high speed with rotor 876 for 50 mL tubes, Cat. No. 20671-007, VWR Scientific.
- n. Syringeless filter device – Mini-UniPrep, 0.45 μ m nylon, Cat. No. UN203NPUNYL, Whatman.
- o. Evaporator – N-Evap, Organomation Associates Inc.
- p. Filter – 0.45 μ m, nylon, acrodisc-13, Cat. No. 4551, Pall Gelman Sciences, Inc.
- q. Analytical balance – 0.0001 g sensitivity, AG204, Mettler.
- r. Volumetric flasks – 1L, 100 mL amber, 10 mL amber.
- s. HPLC mobile phase filtering and degassing apparatus – Microfiltration Assembly, 47 mm, Millipore.
- t. HPLC guard column - C18 Security Guard cartridge, 2.1 x 4 mm, 2 μ m particles, Cat. No. AJO-4286 and Security Guard kit, Cat. No. KJO-4282, Phenomenex.
- u. HPLC column - BetaMax Base, 2.1 x 100 mm, 5 μ m particles, Cat. No. 95105-

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02	Page 5 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

102130, Thermo Hypersil.

2. Instrumentation

- a. Waters Alliance 2695 HPLC equipped with an autosampler.
- b. Waters Micromass Quattro micro API mass spectrometer.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents / solutions may be substituted for the following.

1. Reagents

- a. Methanol (MeOH) – HPLC grade, Cat. No. 230-4, Burdick & Jackson.
- b. Acetonitrile (ACN) – HPLC grade, Cat. No. 015-4, Burdick & Jackson.
- c. Isopropanol (IPA) – HPLC grade, Cat. No. AH323-4, Burdick & Jackson.
- d. Sodium chloride (NaCl) – ACS reagent grade, Cat. No. S271-1, Fisher.
- e. Sodium sulfate (Na₂SO₄) – ACS reagent grade, Cat. No. 354250010, Acros Organics.
- f. Magnesium sulfate (MgSO₄) – anhydrous, minimum 99.5% purity, Cat. No. M-7506, Sigma.
- g. Water – Deionized, HPLC grade, Millipore Rx system.
- h. Formic acid – Purity 98 - 100%, Cat. No. 27001, Riedel-de Haën.

2. Solutions

- a. Aqueous mobile phase (0.1% formic acid in water):
Pipet 1 mL of formic acid into a 1 L volumetric flask. Fill to volume with Millipore water. Degas before use. Vacuum filter if desired through a 0.45 µm nylon filter.

D. STANDARDS

1. Source

- a. Clenbuterol HCl (CLEN) – approximately 95% pure, Cat. No. C-5423, Sigma Chemical Co.
- b. Cimaterol (CIM) – Cat. No. 159757, MP Biochemicals Inc.
- c. Salbutamol (SAL) – approximately 95% pure, Cat. No. S-8260, Sigma Chemical Co.
- d. Ractopamine HCl (RAC) – Elanco Animal Health.
- e. Zilpaterol (ZIL) – Intervet Inc.

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02	Page 6 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

2. Preparation

Note: Adjust all standard weights for purity.

- a. CLEN, CIM, SAL and ZIL stock standards (~25 µg/mL):
Weigh ~2.5 mg of CLEN, CIM, SAL and ZIL into its own 100 mL amber volumetric flask and bring to volume with methanol. Record the weight to 0.1 mg and calculate the exact concentration. These standards are stable for 2 months when stored in the refrigerator at 2 - 8 °C.
- b. RAC stock standard (~1 mg/mL):
Weigh ~10.0 mg into a 10 mL volumetric flask and dilute to volume with methanol. Record the weight to 0.1 mg and calculate the exact concentration. This standard is stable for 3 months when stored in the refrigerator at 2 - 8 °C.
- c. RAC intermediate standard (10 µg/mL):
Pipet ~100 µL (adjusted for the actual stock standard concentration) of the RAC stock standard into a 10 mL volumetric flask and bring to volume with Millipore water. This standard is stable for 1 month when stored in the refrigerator at 2 - 8 °C.
- d. Mixed intermediate standard A (250 ng/mL CLEN, CIM, SAL; 500 ng/mL ZIL; 1.75 µg/mL RAC):
Pipet ~100 µL (adjusted for the actual stock standard concentration) of the CLEN, CIM, SAL stock standards, ~200 µL of the ZIL stock standard, and ~1.75 mL of the 10 µg/mL RAC intermediate standard into a 10 mL amber volumetric flask and bring to volume with Millipore water. This standard is stable for 1 month when stored in the refrigerator at 2 - 8 °C.
- e. Mixed intermediate standard B (50 ng/mL CLEN, CIM, SAL; 100 ng/mL ZIL; 0.35 µg/mL RAC):
Pipet 200 µL of the mixed intermediate standard A into an HPLC vial and add 800 µL Millipore water. This standard is stable for 1 month when stored in the refrigerator at 2 - 8 °C.
- f. Mixed external standard (3 ng/mL CLEN, CIM, SAL; 6 ng/mL ZIL; 21 ng/mL RAC):
Pipet 60 µL of mixed intermediate standard B and 940 µL of Millipore water into an HPLC vial. This standard is stable for 1 month when stored in the refrigerator at 2 - 8 °C.

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02	Page 7 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

E. SAMPLE PREPARATION

1. Retinal excision

- a. Eyeballs should be thawed at room temperature just enough so that the outside tissues can be manipulated but the aqueous and vitreous humors are still frozen. This takes about 45 to 60 minutes. The eyeball should not deform any noticeable amount when squeezed.
- b. Incise the eyeball by slowly cutting across the cornea horizontally and vertically with a scalpel or a razor blade, using whichever provides more control during the cutting process. The length of the incisions should include the full width and length of the cornea, and extend into the sclera to allow the eyeball to be everted. Force the semi-frozen contents (aqueous and vitreous humors and lens) out of the eyeball and retain (the eye contents may be discarded if retinal results are negative for beta agonists).

Caution: To ensure minimal risk from BSE while performing the excision, wear a cut resistant glove on the hand holding the eyeball, and safety glasses or a face shield. Wash hands thoroughly after performing the excision.

- c. Evert the eyeball, and scrape the choroid/PRE layer into a petri dish. This layer is distinctive in bovine species due to its iridescent bluish-green metallic coloration on black background. Include black filmy tissue emanating from the optic nerve area (neural retina) if observed.

Note: Whole eyeballs can be stored at -20 °C or lower for at least one year. Extracted retinal tissue can be stored at -40 °C or lower for one week (longer results in dry tissue).

- d. With a second razor blade, mince the choroid/PRE tissue into fine pieces prior to weighing.

2. Liver and muscle homogenization

- a. Cut liver or muscle sample into smaller pieces and homogenize in a blender or food processor.
- b. Transfer homogenized sample into a plastic bag and store in a freezer at -10 °C or colder.
- c. Let sample partially thaw prior to analysis.

F. ANALYTICAL PROCEDURE

1. Sample Extraction for Retinal Tissue

- a. Weigh 0.4 ± 0.02 g retina into a 50 mL round bottom polyallomer centrifuge tube. Prepare positive and negative controls to be included in the sample set at this

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02	Page 8 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

time. Weigh two blank tissue portions. Fortify one by adding 24 μ L of mixed intermediate standard B (D.2.e).

- b. Add 800 μ L acetonitrile and 200 μ L isopropanol. Vortex for 2 minutes while pounding tissue with a glass rod to mash retinal tissues.
- c. Add 0.24 g NaCl and vortex for 2 minutes.
- d. Add 0.80 g Na₂SO₄ and 0.10 g MgSO₄ and vortex for 2 minutes.

Note: This is a suitable stopping point. Samples may be stored overnight at 2 - 8 °C.

- e. Centrifuge at 15,000 rpm for 10 minutes or sufficiently to pack tissue solid.
- f. Decant the extract into Whatman mini-uniprep filter vial.
- g. Evaporate to dryness with air or nitrogen.
- h. Add 0.4 mL Milli-Q water to vial and filter reconstituted extract by pushing plunger-shaped cap equipped with a 0.45 μ m nylon filter into vial.

2. Sample Extraction for Liver and Muscle Tissue

- a. Weigh 5.0 \pm 0.1 g homogenized liver or muscle into a 50 mL disposable centrifuge tube. Prepare positive and negative controls to be included in the sample set at this time. Weigh two blank tissue portions. Fortify one by adding 60 μ L of the mixed intermediate standard A (D.2.d.).
- b. Add 4 mL acetonitrile and 1 mL isopropanol. For liver, cap and shake or vortex for 2 minutes. For muscle, homogenize using a Tissuemizer for 30 seconds.
- c. Add 1.2 g NaCl and shake or vortex for 2 minutes.
- d. Add 4 g Na₂SO₄ and 0.5 g MgSO₄ and shake or vortex for 2 minutes.

Note: This is a suitable stopping point. Samples may be stored overnight at 2 - 8 °C.

- e. Pipet 0.6 mL of extract into Whatman mini-uniprep filter vial.
- f. Evaporate to dryness with air or nitrogen.
- g. Add 0.6 mL Milli-Q water to vial and filter reconstituted extract by pushing plunger-shaped cap equipped with a 0.45 μ m nylon filter into vial.

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02	Page 9 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

3. LC/MS/MS Analysis

a. Instrument Settings

Note: The following instrument parameters may be optimized:

i. HPLC Conditions:

Aqueous Mobile Phase	0.1% formic acid in water
Organic Mobile Phase	Acetonitrile
Flow Rate	0.3 mL/min
Column Temperature	25 °C
Injection Volume	50 µL
Run Time	12 minutes

ii. HPLC Mobile Phase Gradient Table:

Time	% Aqueous	% Organic
0:00	95%	5%
1:30	95%	5%
7:30	35%	65%
7:36	95%	5%
12:00	95%	5%

iii. Interface Conditions:

Ion Mode	ES+
Source Temperature	125 °C
Desolvation Temperature	400 °C
Cone Gas Flow	25 L/hr
Desolvation Gas Flow	900 L/hr

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02	Page 10 of 26
Title: Identification of Beta-Agonists by HPLC/MS/MS	
Revision: 02	Replaces: CLG-AGON1.01
Effective: 08/27/2007	

iv. MRM Parameters:

	Precursor Ion (m/z)	Product Ion (m/z)*	Collision Energy (eV)
Salbutamol	240	148	18
		166	14
		222	11
Cimaterol	220	116	33
		143	23
		160	16
Clenbuterol	277	168	32
		203	15
		259	11
Ractopamine	302	107	27
		121	21
		164	16
Zilpaterol	262	157	33
		185	25
		244	13

* Most abundant product ion is in bold.

v. MS Parameters:

Segment #	1	2
Starting Retention Time (min)	0.0	4.0
Dwell Time (sec)	0.2	0.2
Capillary (kV)	1	1
Multiplier (V)	650	650
Analytes	SAL, CIM, & ZIL	CLEN & RAC

4. Sample Chromatograms

- a. Sample Chromatograms and Mass Spectra, see Appendix K. 3
- b. Proposed fragmentation patterns, see Appendix, K.2.

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02	Page 11 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

G. IDENTIFICATION

1. The retention time of each analyte must match that of the external standard within 5%.
2. LC/MS/MS Criteria

Product ion abundance ratios for each analyte must match that of the external standard within 20% relative. The required ion ratios are as follows. Alternatively, the reciprocals may be used.

	Ratio #1	Ratio #2
SAL	148 / 166	148 / 222
CIM	160 / 116	160 / 143
CLEN	203 / 168	203 / 259
RAC	164 / 121	164 / 107
ZIL	244 / 185	244 / 157

3. The following injection sequence may be used for sample analysis:
 - a. External standard
 - b. Solvent blank
 - c. Negative tissue control
 - d. Positive tissue control
 - e. Sample(s)
 - f. External standard
4. The water blank analyzed after the external standard must be negative for all analytes.
5. The tissue blank must be negative for all analytes.
6. The tissue fortification must be positive for all analytes.

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02	Page 12 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment — safety glasses and/or face shield, disposable gloves, cut resistant gloves, lab coat.
2. Hazards

<i>Procedure Step</i>	<i>Hazard</i>	<i>Recommended Safe Procedures</i>
Preparation of retinal tissue using scalpel/razor blade	May cause accidental injury to hands	Use cut resistant gloves and other protective equipment
Methanol, acetonitrile, and isopropanol	Flammable and poisonous	Use reagents in an efficient fume hood away from all electrical devices and open flames. Wear gloves and protective eyewear.
Formic acid	Acid burns	Wear protective equipment and avoid contact with skin.

3. Disposal Procedures

<i>Procedure Step</i>	<i>Hazard</i>	<i>Recommended Safe Procedures</i>
Methanol, acetonitrile, and isopropanol	Flammable and poisonous	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.
Formic acid	Acid burns	Collect waste in tightly sealed container and store away from non-compatibles in a cool, well ventilated, acid storage area/cabinet for disposal in accordance with local, state, and federal regulations.

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02	Page 13 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

I. QUALITY ASSURANCE PLAN

1. Performance Standard

- a. Positive control is positive for all analytes using the criteria in Section G.
- b. Negative control is negative for all analytes using the criteria in Section G..

2. Critical Control Points and Specifications

	<i>Record</i>	<i>Acceptable Control</i>
a. Sample weight of retinal tissue		0.4 ± 0.02 g
b. Sample weight of liver or muscle tissue		5.0 ± 0.1 g

3. Readiness To Perform

a. Familiarization

- i. Phase I: Analyze an external standard (D.2.f) and a solvent blank over 3 days to ensure that instrument response is adequate for confirmation.
- ii. Phase II: Analyze 1 blank and 4 fortified samples over at least 3 different days (at least 1 set for each tissue of interest).

Note 1: When using retinal tissue, these will not be from the same animal due to sample size constraints.

Note 2: Phase I and Phase II may be performed concurrently.

- iii. Phase III: Check samples for analyst accreditation.
 - (a) Each tissue of interest must have at least three samples: at least one must be negative, and at least one must be fortified at the level of interest . All samples must be blind to the analyst.
 - (b) Report analytical findings to Supervisor/Quality Assurance Manager (QAM).
 - (c) Letter from QAM is required to commence official analysis.

b. Acceptability criteria

Refer to I. 1.

4. Intralaboratory Check Samples

a. System, minimum contents.

- i. Frequency: One per week per analyst when samples analyzed.

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02	Page 14 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

- ii. Records are to be maintained for review.
 - b. Acceptability criteria.
Refer to I. 1.
If unacceptable values are obtained, then:
 - i. Stop all official analyses by that analyst.
 - ii. Take corrective action.
- 5. Sample Acceptability and Stability
 - a. Retinal tissue
 - i. Bovine PRE/choroid retinal layer
 - ii. Sample storage: Whole eyeballs should be stored at -20 °C or lower and can be kept for at least one year under these conditions. Excised retinal tissue should be stored at -40 °C or lower and can be stored for one week.
 - b. Liver and muscle tissue
 - i. Bovine, porcine, ovine, and caprine liver; bovine and porcine muscle
 - ii. Sample storage: Homogenized liver and muscle should be stored at -10 °C or lower and can be kept for at least two months under these conditions.
- 6. Sample Set
 - a. Negative control (tissue blank).
 - b. Positive control (fortified blank).
 - c. Test samples to be analyzed.
- 7. Sensitivity
 - a. Minimum proficiency level (MPL):

Analyte	MPL (ppb)
Clenbuterol	3
Salbutamol	3
Cimaterol	3
Zilpaterol	6
Ractopamine	21

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02	Page 15 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

J. WORKSHEET

Following are example worksheets.

Beta Agonist LC/MS/MS Worksheet

Analyst	
Start Date	
End Date	
Peer Review/Date	
Supervisor Review/Date	

Tissue Type	Retina / Liver / Muscle
LC/MS/MS	
LC Sample List	
Vortex / Shaker	
Centrifuge	
Tissuemizer	
Evaporator	
Sample Freezer	
Sample Balance	

Reagents	ID	Pipet/Balance
Acetonitrile (800 µL)		
Isopropanol (200 µL)		
NaCl (0.24 g)		
Na ₂ SO ₄ (0.80 g)		
MgSO ₄ (0.10 g)		
MilliQ Water (400/600 µL)	n/a	
Aqueous mobile phase		n/a
Standards		
External Standard		n/a
Fortification Solutions(s):		
(24 µL mixed 50 ppb std/ 60 µL individual 250 ppb std)		

Sample	ID	Weight	Sample	ID	Weight
1			17		
2			18		
3			19		
4			20		
5			21		
6			22		
7			23		
8			24		
9			25		
10			26		
11			27		
12			28		
13			29		
14			30		
15			31		
16			32		

Comments:

**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02	Page 18 of 26
Title: Identification of Beta-Agonists by HPLC/MS/MS	
Revision: 02	Replaces: CLG-AGON1.01
	Effective: 08/27/2007

K. APPENDIX

1. Proposed fragmentation patterns

Clenbuterol

Ion (m/z)	Fragment
277	M^+
259	$[M^+-H_2O]^+$
203	$[M^+-H_2O-(CH_3)_2C=CH_2]^+$
168	$[M^+-H_2O-(CH_3)_2C=CH_2-Cl]^+$

Salbutamol

Ion (m/z)	Fragment
240	$[M+H]^+$
222	$[M+H-H_2O]^+$
166	$[M+H-H_2O-(CH_3)_2C=CH_2]^+$
148	$[M+H-2H_2O-(CH_3)_2C=CH_2]^+$

Cimaterol

Ion (m/z)	Fragment
220	$[M+H]^+$
160	$[M+H-H_2O-CH_3CH=CH_2]^+$
143	$[M+H-H_2O-(CH_3)_2CHNH_2]^+$
116	$[M+H-H_2O-(CH_3)_2CHNH_2-HCN]^+$

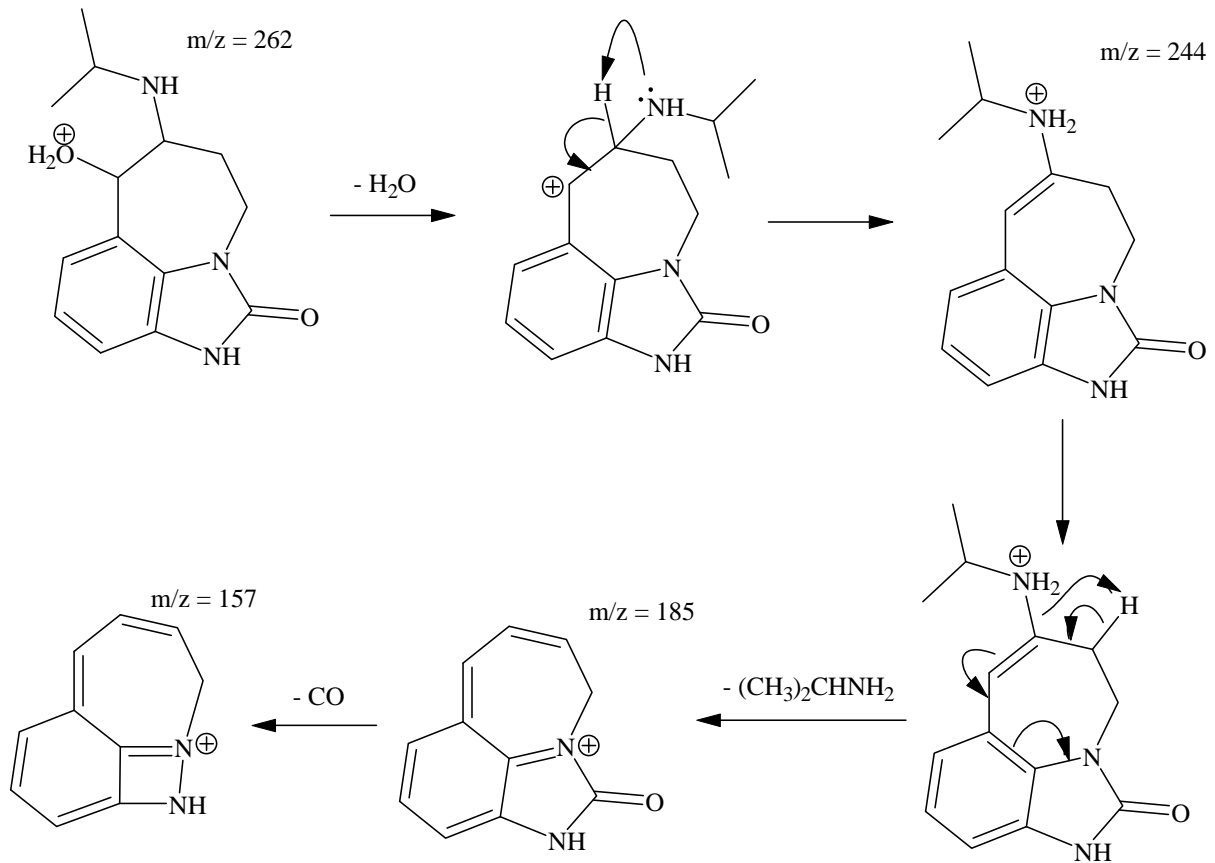
Ractopamine

Ion (m/z)	Fragment
302	$[M+H]^+$
164	$[M+H-CH_2CHOH(C_6H_4)OH]^+$
121	$[M+H-(CH_3)CHNHCH_2CHOH(C_6H_4)OH]^+$
107	$[M+H-CH_2CH(CH_3)NHCH_2CHOH(C_6H_4)OH]^+$

United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science

CLG-AGON1.02	Page 19 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

Zilpaterol

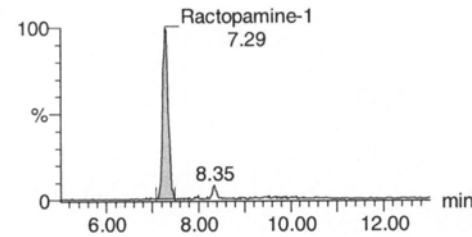
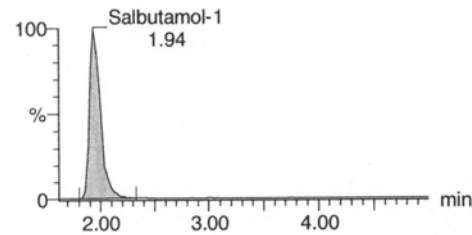
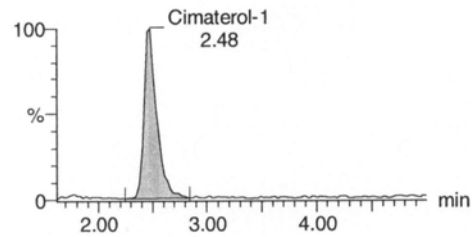
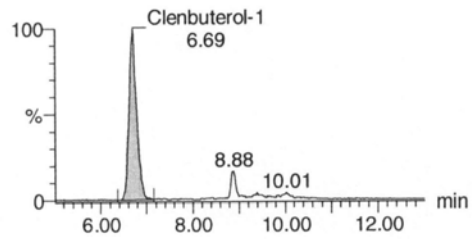


**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02	Page 20 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

2. Sample Chromatograms and Mass Spectra

a. Bovine Fortified Retina, 3 ppb

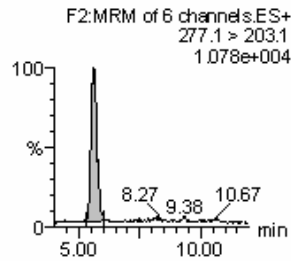


**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

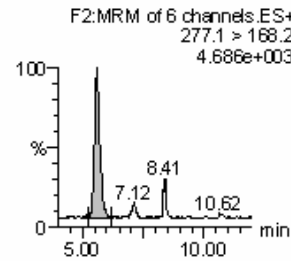
CLG-AGON1.02	Page 21 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

b. Bovine Fortified Liver, 3 ppb

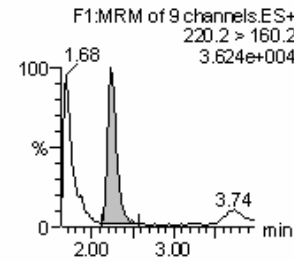
Clenbuterol-1



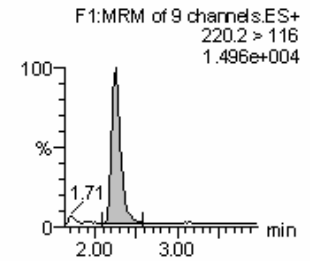
Clenbuterol-1



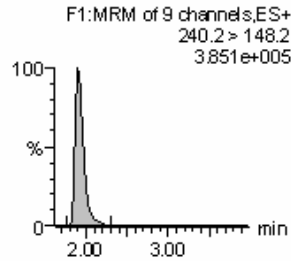
Cimaterol-1



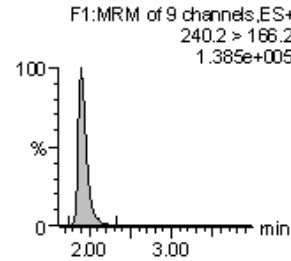
Cimaterol-1



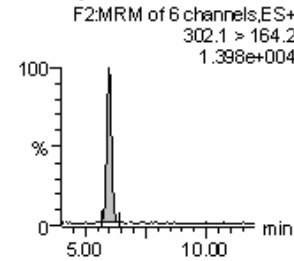
Salbutamol-1



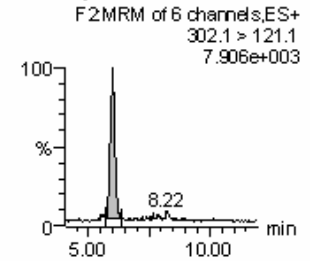
Salbutamol-1



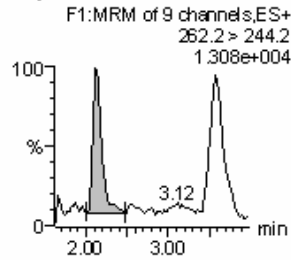
Ractopamine-1



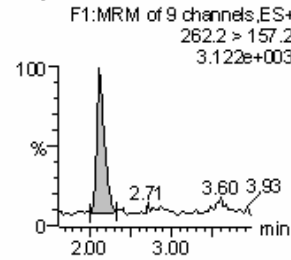
Ractopamine-1



Zilpaterol-1



Zilpaterol-1

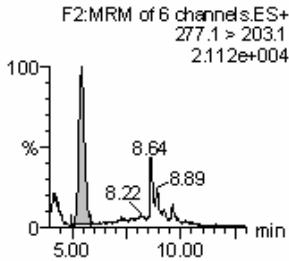


**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

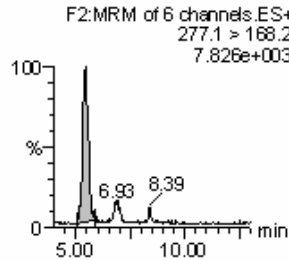
CLG-AGON1.02		Page 22 of 26
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

c. Porcine Fortified Liver, 3 ppb

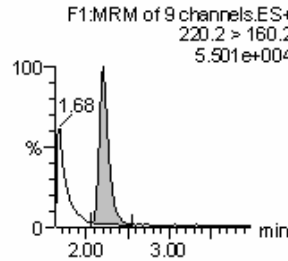
Clenbuterol-1



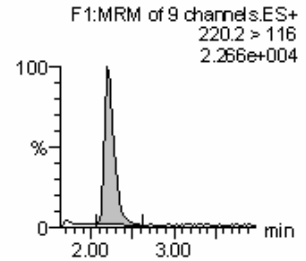
Clenbuterol-1



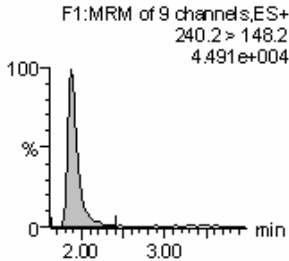
Cimaterol-1



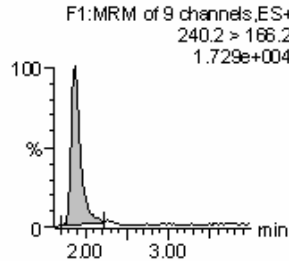
Cimaterol-1



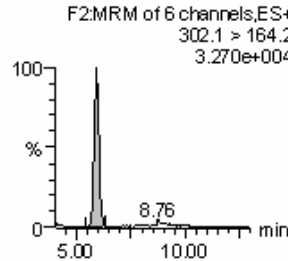
Salbutamol-1



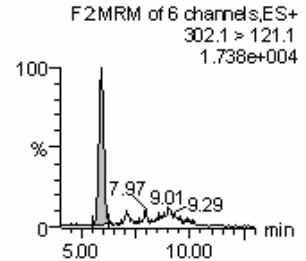
Salbutamol-1



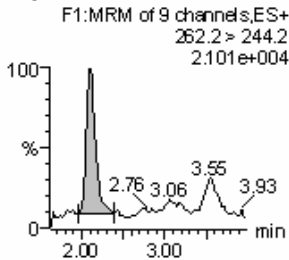
Ractopamine-1



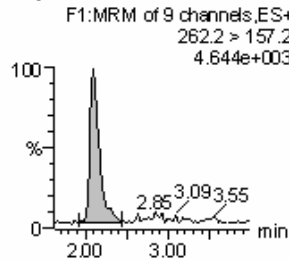
Ractopamine-1



Zilpaterol-1



Zilpaterol-1

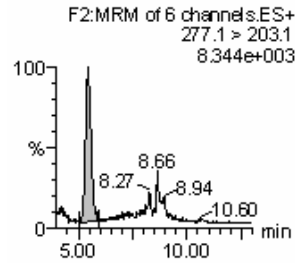


**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

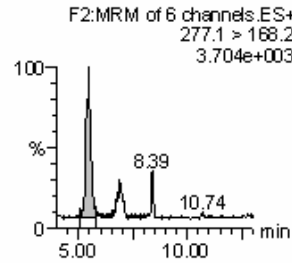
CLG-AGON1.02		Page 23 of 26
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

d. Ovine Fortified Liver, 3 ppb

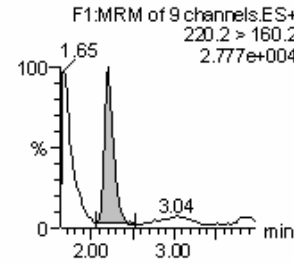
Clenbuterol-1



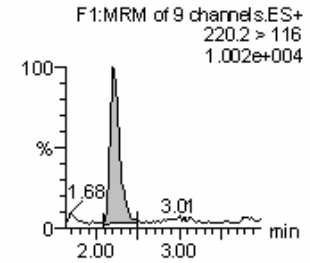
Clenbuterol-1



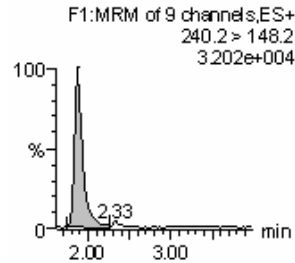
Cimaterol-1



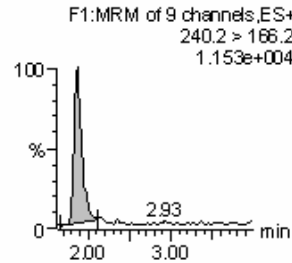
Cimaterol-1



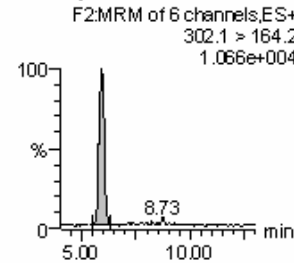
Salbutamol-1



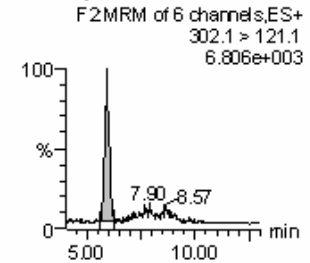
Salbutamol-1



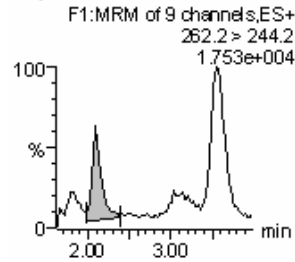
Ractopamine-1



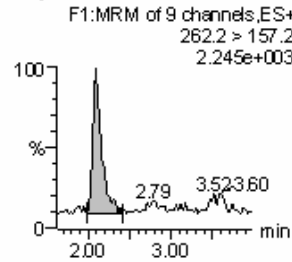
Ractopamine-1



Zilpaterol-1



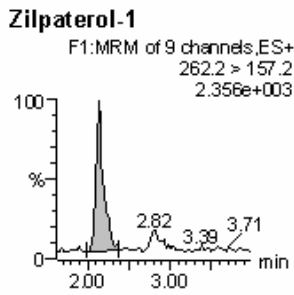
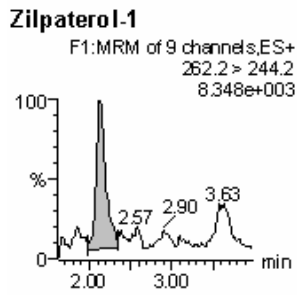
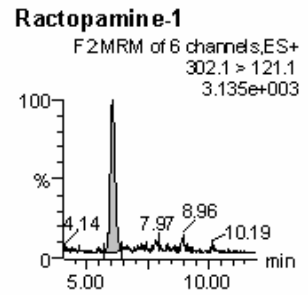
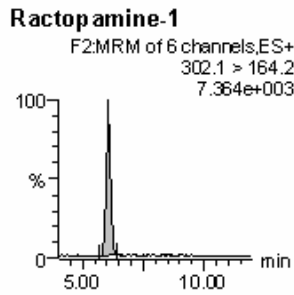
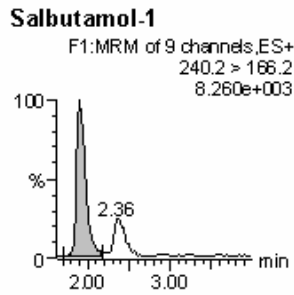
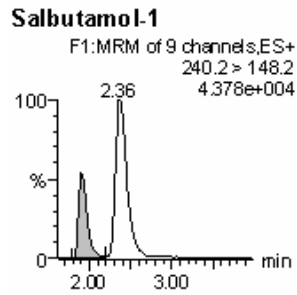
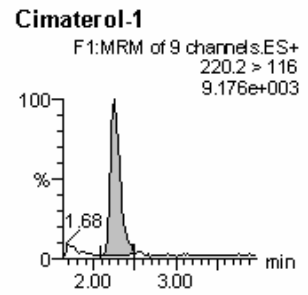
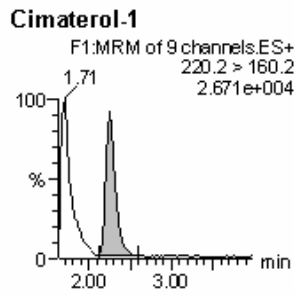
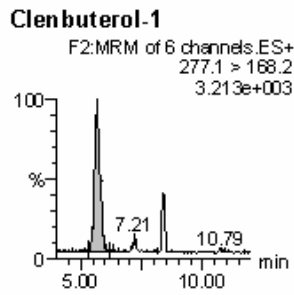
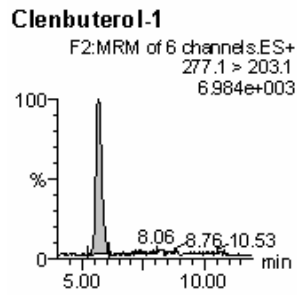
Zilpaterol-1



**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02		Page 24 of 26
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

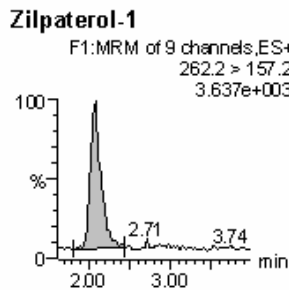
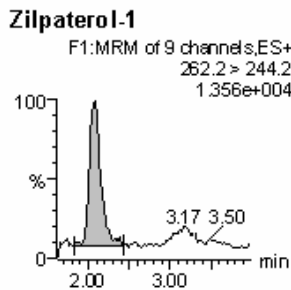
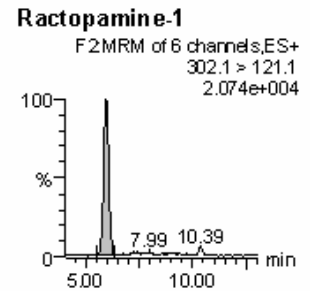
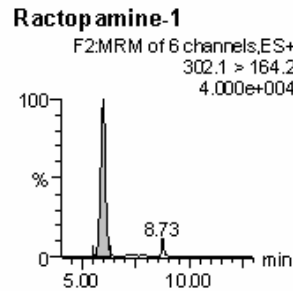
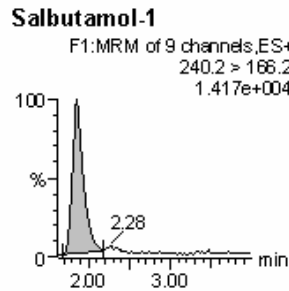
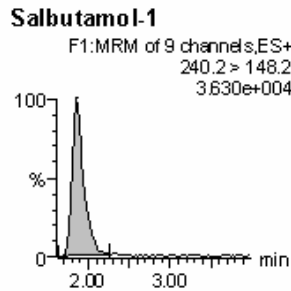
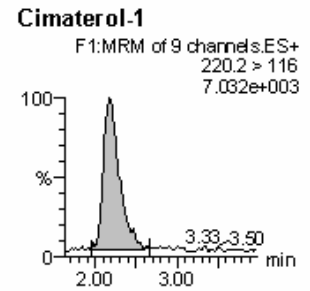
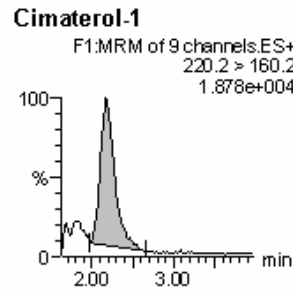
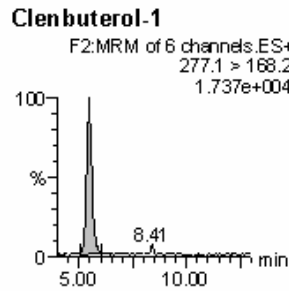
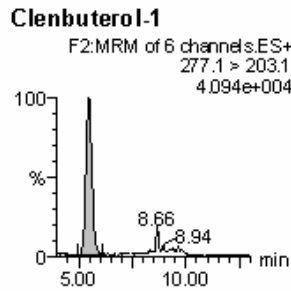
e. Caprine Fortified Liver, 3 ppb



**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

CLG-AGON1.02	Page 25 of 26
Title: Identification of Beta-Agonists by HPLC/MS/MS	
Revision: 02	Replaces: CLG-AGON1.01
	Effective: 08/27/2007

f. Bovine Fortified Muscle, 3 ppb

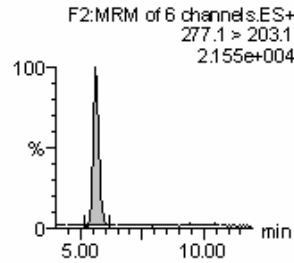


**United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science**

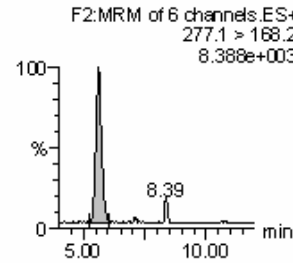
CLG-AGON1.02	Page 26 of 26	
Title: Identification of Beta-Agonists by HPLC/MS/MS		
Revision: 02	Replaces: CLG-AGON1.01	Effective: 08/27/2007

g. Porcine Fortified Muscle, 3 ppb

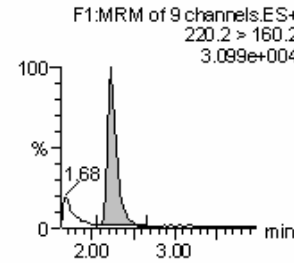
Clenbuterol-1



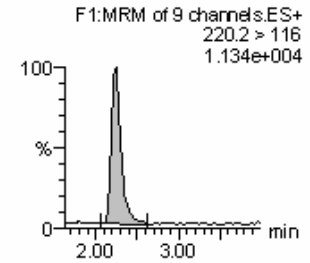
Clenbuterol-1



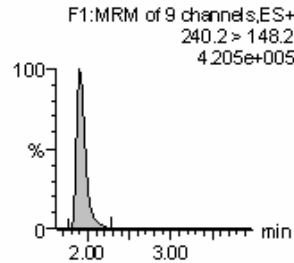
Cimaterol-1



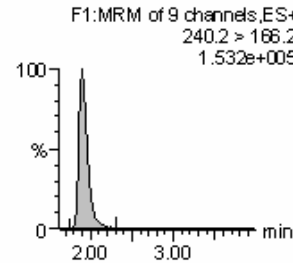
Cimaterol-1



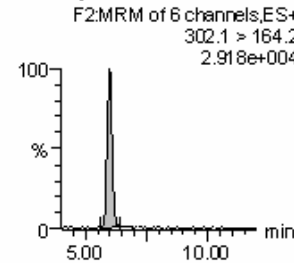
Salbutamol-1



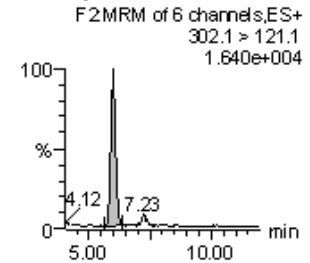
Salbutamol-1



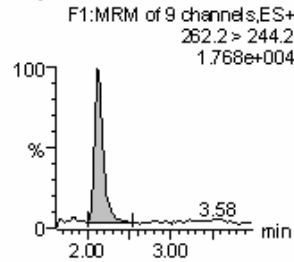
Ractopamine-1



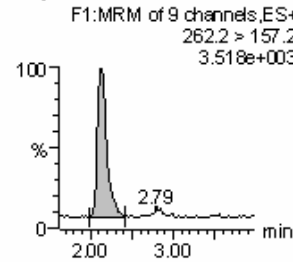
Ractopamine-1



Zilpaterol-1



Zilpaterol-1



L. APPROVALS AND AUTHORITIES

1. Approvals on file.
2. Issuing Authority: Laboratory Quality Assurance Division.