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Title: Screening and Confirmation of Beta-Agonists by HPLC/MS/MS		
Revision: 04	Replaces: CLG- AGON1.03	Effective: 07/02/2012

Contents

A. INTRODUCTION

1. Background

Beta-agonists are also known as beta-adrenergic agonists. An adrenergic agent is a drug that has effects similar to epinephrine (adrenaline). Adrenergic drugs either stimulate a response (agonists) or inhibit a response (antagonists). The five categories of adrenergic receptors are: α_1, α_2, β_1, β_2, and β_3, and agonists vary in specificity between these receptors, and may be classified respectively. Thus, beta-agonists stimulate a response of the beta receptors.

Beta-agonists are used for growth promotion in food animals, increasing lean muscle mass. In humans, clenbuterol and salbutamol are used as bronchodilators by asthma sufferers and as performance-enhancing drugs by athletes. Human side effects include increased heart rate and blood pressure, anxiety, palpitation, and skeletal muscle tremors.

2. Summary of Procedure

Free residues of clenbuterol, salbutamol, cimaterol, zilpaterol, and ractopamine are extracted from retinal, liver, or muscle tissues with a mixture of acetonitrile and isopropanol. Sodium chloride, sodium sulfate, and magnesium sulfate salts are used to precipitate proteins and dehydrate the solution. This extract is evaporated, reconstituted in water, filtered, and analyzed by HPLC/MS/MS.

3. Applicability

This method is suitable for the screening and/or confirmation of β-agonists in bovine retinal tissue (except for zilpaterol); bovine, porcine, ovine, and caprine liver; and bovine and porcine muscle. Screening is applicable at ≥ 3 ppb for clenbuterol, salbutamol, cimaterol, and ractopamine ● HCl; and ≥ 6 ppb for zilpaterol.

Note: Refer to 21CFR for tolerance values set by FDA.

B. EQUIPMENT

Note: Equivalent equipment may be substituted.

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, Kinematica AG, Model No. PT2100.

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. Micropipettes – Adjustable, 10 – 5000 µL, 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 µm nylon, Cat. No. UN203NPUNYL, Whatman.

o. Evaporator – N-Evap 112, Organomation Associates Inc., Model No. 8125.

p. Filter – 0.45 µm, nylon, acrodisc-13, Cat. No. 4551, Pall Gelman Sciences, Inc.

q. Amber autosampler vial, 12 x 32 mm, E&K, Cat. No. E251011

r. Analytical balance – 0.0001 g sensitivity, AG204, Mettler.

s. Volumetric flasks – 1 L, 100 mL amber, 10 mL amber.

t. HPLC mobile phase filtering and degassing apparatus – Microfiltration Assembly, 47 mm, Millipore.

u. HPLC guard column - C18 Security Guard cartridge, 2.1 x 4 mm, 2 µm particles, Cat. No. AJO-4286 and Security Guard kit, Cat. No. KJO-4282, Phenomenex.

v. HPLC column - BetaMax Base, 2.1 x 100 mm, 5 µm particles, Cat. No. 95105-102130, Thermo Hypersil.

w. Food processor – Robot Coupe model RSI6Y-1, Robot Coupe USA Inc.

x. Sample cups – eValue 4.5 oz specimen containers with caps, Cat. No. C686550, E&K Scientific.

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. The stability time frame of the solution is dependant on the expiration date of the components used or the listed expiration date, whichever is soonest.

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 (Na_2SO_4) – ACS reagent grade, Cat. No. 354250010, Acros Organics.

   f. Magnesium sulfate ($MgSO_4$) – 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):

      Pipette 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. This solution is stable for 1 month at room temperature.

D. STANDARD(S)

Note: Equivalent standards / solutions may be substituted. The stability time frame of the solution is dependant on the expiration date of the components used or the listed expiration date, whichever ends sooner.

1. Standard Information

   a. Clenbuterol (CLEN) ● HCl– 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.

2. Preparation of Standard Solution(s)

Note: Adjust all standard weights for purity. The concentration of Clenbuterol ● HCl is to be corrected for salt, since Clenbuterol is the analyte of interest as opposed to Clenbuterol ● HCl. The Ractopamine ● HCl concentration is not to be corrected for salt since Ractopamine ● HCl is the analyte of interest. See calculation in section G.

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 a 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 a refrigerator at 2 - 8 °C.

c. RAC intermediate standard (10 µg/mL):

Pipette ~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 a refrigerator at 2 - 8 °C.

d. Mixed intermediate standard A (250 ng/mL CLEN, CIM, SAL, RAC; 500 ng/mL ZIL):

Pipette ~100 µL (adjusted for the actual stock standard concentration) of the CLEN, CIM, SAL stock standards, ~200 µL of the ZIL stock standard, and ~250 µL 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 a refrigerator at 2 - 8 °C.

e. Mixed intermediate standard B (50 ng/mL CLEN, CIM, SAL, RAC; 100 ng/mL ZIL):

Pipette 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 a refrigerator at 2 - 8 °C.

f. Mixed external standard (3 ng/mL CLEN, CIM, SAL, RAC; 6 ng/mL ZIL):

Pipette 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 a refrigerator at 2 - 8 °C.

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 ºC or lower for at least one year. Extracted retinal tissue can be stored at -40 ºC 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 (For alternative muscle sample homogenization, see E.2.b.)

a. Blender or food processer

i. Cut liver or muscle sample into smaller pieces and homogenize in a blender or food processor.

ii. Transfer homogenized sample into a plastic bag and store in a freezer at -10 ºC or colder.

iii. Let sample partially thaw prior to analysis.

b. Alternatively, dry ice grinding can be used for muscle sample homogenization

i. Chop 0.5 - 1 lb of muscle tissue into small pieces and homogenize with an equal amount of dry ice in a large food processor. The resulting sample homogenate will be a frozen powder.

ii. Transfer a portion of the homogenized sample into a loosely capped sample cup until the dry ice has sublimed. Excess sample from step b.i. may be discarded.

iii. Tighten the caps and store in a freezer at -10 ºC or colder.

F. ANALYTICAL PROCEDURE

1. Preparation of Controls and Samples for Retinal Tissue.

a. Weigh two - 0.4 ± 0.02 g blank retina tissue potions into 50 mL round bottom polyallomer centrifuge tubes.

b. Prepare positive and negative controls by fortifying one with 24 µL of mixed intermediate standard B (D.2.e).

2. Sample Extraction for Retinal Tissue

a. Weigh 0.4 ± 0.02 g retina into a 50 mL round bottom polyallomer centrifuge tube. Include the prepared positive and negative controls in the sample set at this time.

b. Add 800 µL acetonitrile and 200 µL 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 Na_2SO_4 and 0.10 g $MgSO_4$ and vortex for 2 minutes.

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

e. Centrifuge the samples for about five minutes at approximately 2000 RCF.

f. Filter extract by either of:

i. Whatman mini-uniprep filter vial.

(a) Pipette 0.5 mL of extract into Whatman mini-uniprep filter vial.

(b) Evaporate to dryness with air or nitrogen.

(c) Add 0.5 mL Millipore water to vial and filter reconstituted extract by pushing plunger-shaped cap equipped with a 0.45 µm nylon filter into vial.

ii. 3 mL syringe and 0.45 µm nylon filter.

(a) Pipette 0.8 mL of extract into a 12x75mm glass tube.

(b) Evaporate to dryness with air or nitrogen.

(c) Add 0.8 mL of Milli-Q water to the glass tube, vortex for 30 seconds and filter using a 3 mL syringe and 0.45 µm nylon filter into a vial.

3. Preparation of Controls and Samples for Liver and Muscle Tissue.

a. Weigh two – 5.0 ± 0.1 g blank homogenized liver or muscle tissue portions into 50 mL disposable centrifuge tubes.

b. Prepare positive and negative controls by fortifying one with 60 µL of the mixed intermediate standard A (D.2.d.).

4. Sample Extraction for Liver and Muscle Tissue

a. Weigh 5.0 ± 0.1 g homogenized liver or muscle into a 50 mL disposable centrifuge tube. Include the prepared positive and negative controls in the sample set at this time.

b. Add 4 mL acetonitrile and 1 mL isopropanol. For liver and dry ice ground muscle (E.2.b.), cap and shake or vortex for 2 minutes. For muscle homogenized without dry ice (E.2.a), tissuemize for 30 seconds.

c. Add 1.2 g NaCl and shake or vortex for 2 minutes.

d. Add 4 g Na_2SO_4 and 0.5 g $MgSO_4$ and shake or vortex for 2 minutes.

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

e. Centrifuge the samples for about five minutes at approximately 2000 RCF.

f. Filter extract by either of:

i. Whatman mini-uniprep filter vial.

(a) Pipette 0.5 mL of extract into Whatman mini-uniprep filter vial.

(b) Evaporate to dryness with air or nitrogen.

(c) Add 0.5 mL Millipore water to vial and filter reconstituted extract by pushing plunger-shaped cap equipped with a 0.45 µm nylon filter into vial.

ii. 3 mL syringe and 0.45 µm nylon filter.

(a) Pipette 0.8 mL of extract into a 12x75 mm glass tube.

(b) Evaporate to dryness with air or nitrogen.

(c) Add 0.8 mL of Milli-Q water to the glass tube, vortex for 30 seconds and filter using a 3 mL syringe and 0.45 µm nylon filter into a vial.

5. Instrumental Settings

Note: The instrument parameters may be optimized to ensure system suitability.

a. 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

b. 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%

c. 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

d. 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.

e. 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

6. Injection sequence/Sample Set
   a. External Standard
   b. Positive Control
   c. Water Blank
   d. Negative Control
   e. Intra-laboratory check sample (if needed)
   f. Samples, up to a maximum of 48

   The external standard, positive control and negative control must be re-injected after a maximum of 24 samples. The positive control shall also be re-injected at the end of the injection sequence.

G. CALCULATIONS / IDENTIFICATION

1. Calculations

   a. Correction of Clenbuterol Concentration for Salt and Purity

$$\text{Mass}_{\text{Clenbuterol}} = \text{Mass}_{\text{Clenbuterol} \bullet \text{HCl}} \times \frac{\text{Molecular Mass}_{\text{Clenbuterol}}}{\text{Molecular Mass}_{\text{Clenbuterol} \bullet \text{HCl}}} \times \text{Purity}$$

   Where $\text{Mass}_{\text{Clenbuterol} \bullet \text{HCl}}$ = ~2.5 mg

   $\text{Molecular Mass}_{\text{Clenbuterol}}$ = 277.19 g/mol

   $\text{Molecular Mass}_{\text{Clenbuterol} \bullet \text{HCl}}$ = 313.65 g/mol

   Purity = 0.95 (for the product listed)

   b. Estimated Amount Found

   This is a quantitative estimate calculated for comparison to the screen cutoff level. It is based on a one point calibration with the positive control injected most recently before the sample as the reference. The MS instruments can be programmed to automatically do this calculation.

   D = E * B sample / B pos. ctrl.

   Where D = Estimated Amount Found in the Sample (ppb)

   E = Positive Control Fortification Level (ppb)

   B sample = Quant Ion Peak Area in the Sample (counts)

B pos. ctrl. = Quant Ion Peak Area in the Positive Control injected most recently before the sample (counts)

c. Screen Cutoff Level

This level is used to determine if a sample is screen positive or negative. It includes two safety factors so that potential violations are not missed.

$F = 0.5 * G * H$

Where F = Screen Cutoff Level (ppb)

G = Tolerance (ppb)

The tolerance or action level will need to be inserted for the analyte in the product of interest.

For zero and no tolerance situations, samples are screened at the positive control fortification level, which makes $G = 2 * E$, and $F = E * H$.

Screening at half tolerance is the first safety factor.

H = Minimum / Maximum Recovery (unitless)

Take values from Table 1 below.

Note: Values may be updated as more data becomes available.

This is the second safety factor to ensure that violations are not missed due to variation in recovery. These values are based on the positive control having the maximum recovery and the sample having the minimum recovery.

Table 1 – Minimum / Maximum Recovery Values

Cmpd #	**Name**	**Min / Max recovery**
1	Clenbuterol	0.08
2	Cimaterol	0.34
3	Salbutamol	0.40
4	Ractopamine ● HCl	0.31
5	Zilpaterol	0.50

2. Screening Criteria

   a. The retention time of each analyte must match that of the positive control or the external standard injected most recently before the sample within 5%.

   b. All product ions for a given analyte must be present. The required ions are listed in F.5.d.

   c. Each ion must have a signal-to-noise ratio ≥ 3.

   d. The sample is screen positive if the estimated amount found equals or exceeds the screen cutoff level (D ≥ F).

   e. A water blank injected immediately after the initial positive control injection must be negative for all analytes according to criteria G.2.a.-c. above.

   f. Referring to the negative and positive controls injected most recently before the relevant sample, the negative control must be negative for all analytes according to criteria a.-c. above, with the quant ion peak area being ≤ 5% of the positive control injection.

   g. The positive controls injected closest in time before and after the relevant sample must both be positive for all analytes according to criteria b.-c. above and match the retention time of the external standard injected most recently before the relevant sample within 5%.

3. Confirmation Criteria

   a. The retention time must match that of the positive control or the external standard injected most recently before the relevant sample within 5%.

   b. Product ion abundance ratios must match that of the positive control or the external standard injected most recently before the relevant sample within a 20% relative difference. The following are representative ion ratios calculated relative to the most abundant product ion:

	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

   c. Each ion must have a signal-to-noise ratio ≥ 3.

   d. A water blank injected immediately after the initial positive control injection must be negative according to screening criteria G.2.a.-c. above.

e. Referring to the negative and positive controls injected most recently before the relevant sample, the negative control must be negative according to criteria a.-b. above, with the quant ion peak area being ≤ 5% of the positive control.

f. The positive controls injected closest in time before and after the relevant sample must both be positive according to screening criteria G.2.b.-c. above and match the retention time of the external standard injected most recently before the relevant sample set within 5%.

Note: Confirmation criteria for the negative control and positive control are required only for analytes that are to be confirmed in the sample set.

H. SAFETY INFORMATION AND PRECAUTIONS

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

2. Hazards

Procedure Step	*Hazard*	*Recommended Safe Procedures*
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

Follow local, state and federal guidelines for disposal.

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

Record	*Acceptable Control*
Sample weight of retinal tissue	0.4 ± 0.02 g
Sample weight of liver or muscle tissue	5.0 ± 0.1 g

3. Intralaboratory Check Samples
   a. System, minimum contents.
      i. Frequency: One per week per analyst when samples analyzed.
      ii. Records are to be maintained.
   b. Acceptability criteria.
      Refer to I. 1.
      If unacceptable values are obtained, then:
      i. Investigate following established procedures.
      ii. Take corrective action as warranted.

4. Condition upon receipt
   a. Liver, muscle, and retinal tissue – Cold, not spoiled or rancid

J. APPENDIX

1. Proposed fragmentation patterns

Clenbuterol

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

Salbutamol

Ion (m/z)	Fragment
240	$[M+H]^+$
222	$[M^{\bullet}-H_2O]^+$
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

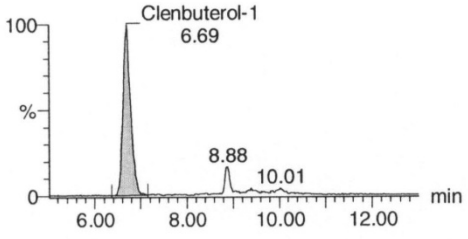
Ion (m/z)	Fragment
302	$[M+H]^+$
164	$[M+H-CH_2CHOH(C_6H_4)OH]^+$
121	$[M+H-(CH_3)CHNHCH_2CHOH(C_6H_4)OH]^+$
107	$[M+H-CH_2CH(CH_3)NHCH_2CHOH(C_6H_4)OH]^+$

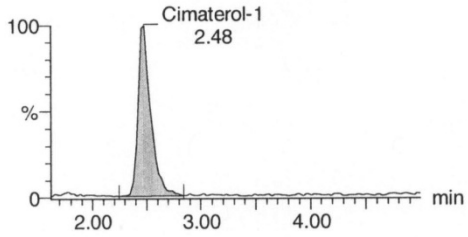
Zilpaterol

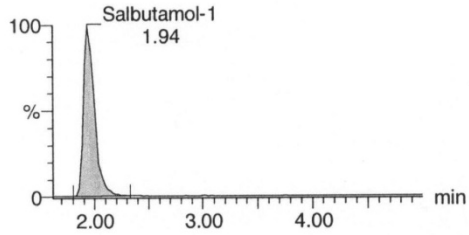
Ion (m/z)	Fragment
262	$[M+H]^+$
244	$[M^{\bullet}-H_2O]^+$
185	$[M+H-H_2O-(CH_3)_2CHNH_2]^+$
157	$[M+H- H_2O-(CH_3)_2CHNH_2-CO]^+$

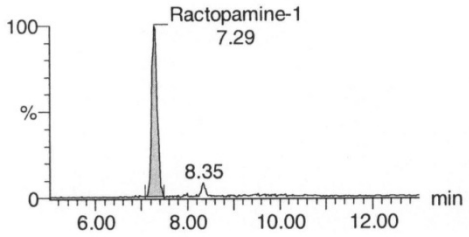
2. Sample Chromatograms and Mass Spectra

   a. Bovine Fortified Retina, 3 ppb

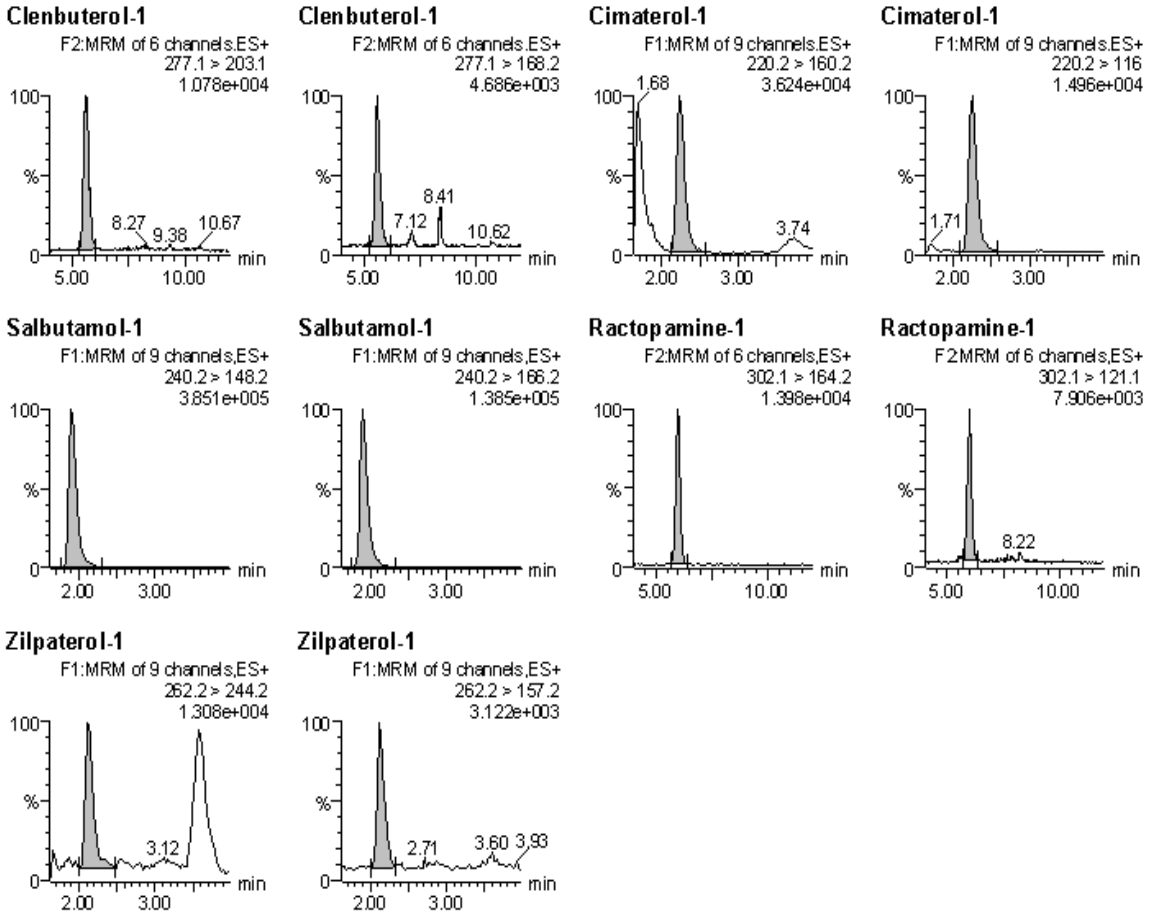




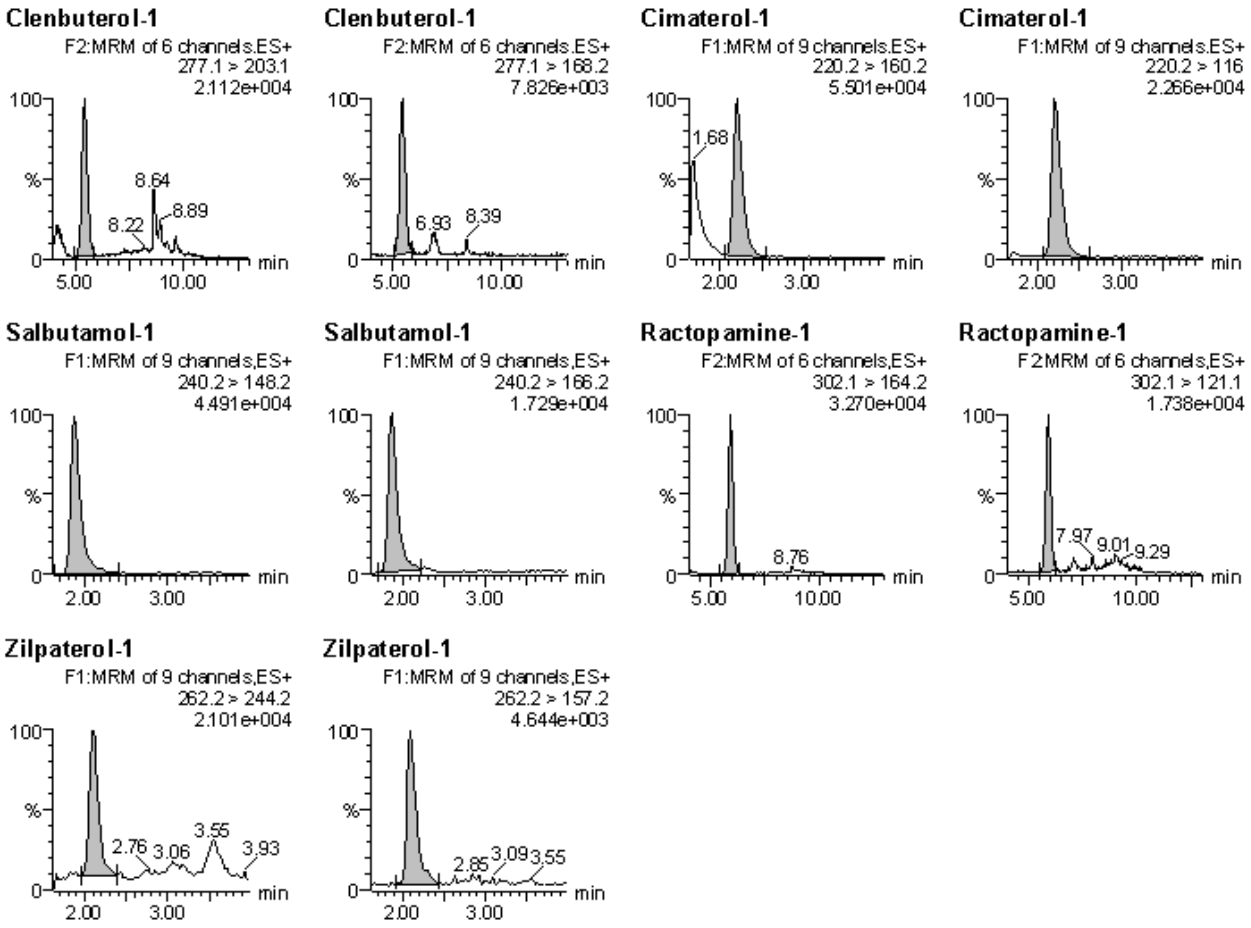




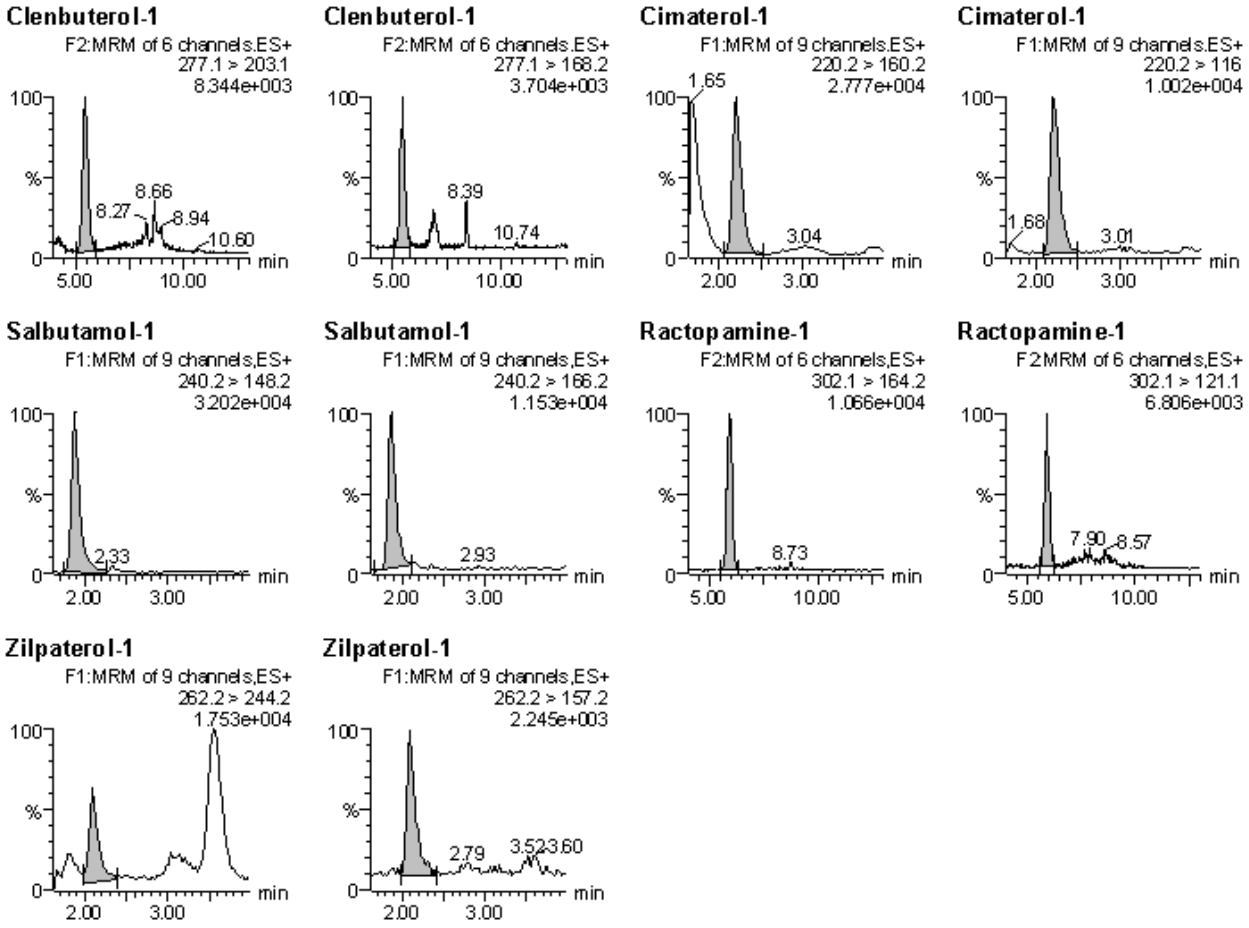
b. Bovine Fortified Liver, 3 ppb



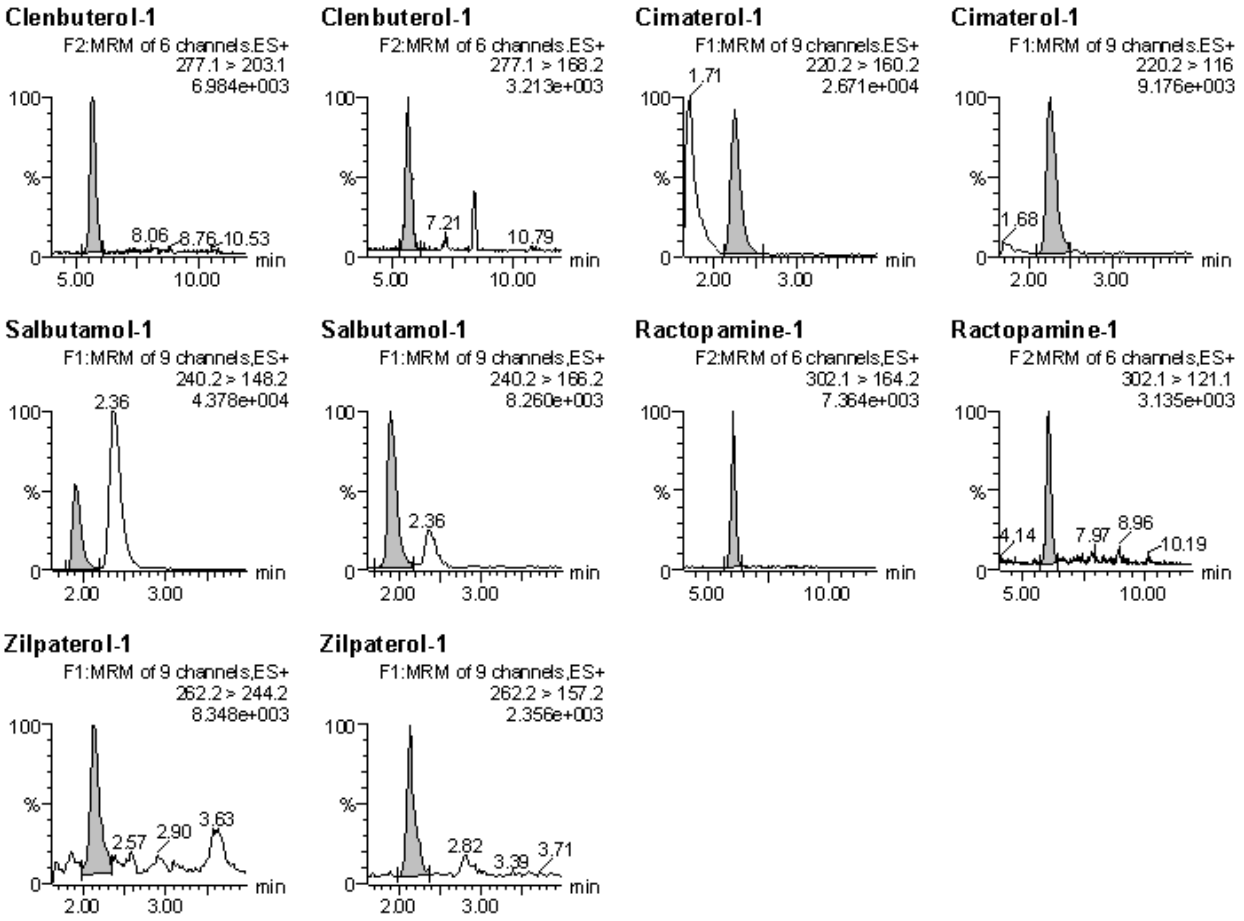
c. Porcine Fortified Liver, 3 ppb



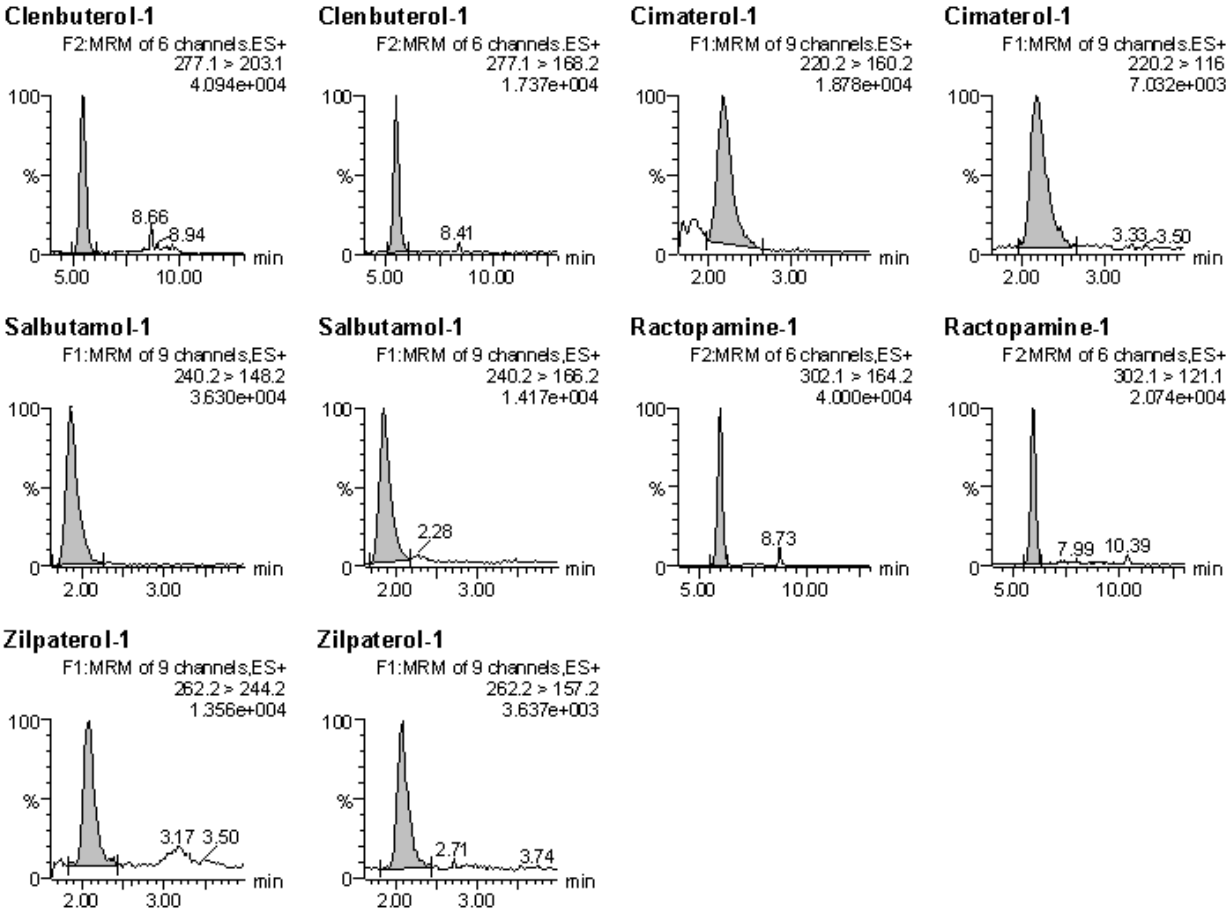
d. Ovine Fortified Liver, 3 ppb



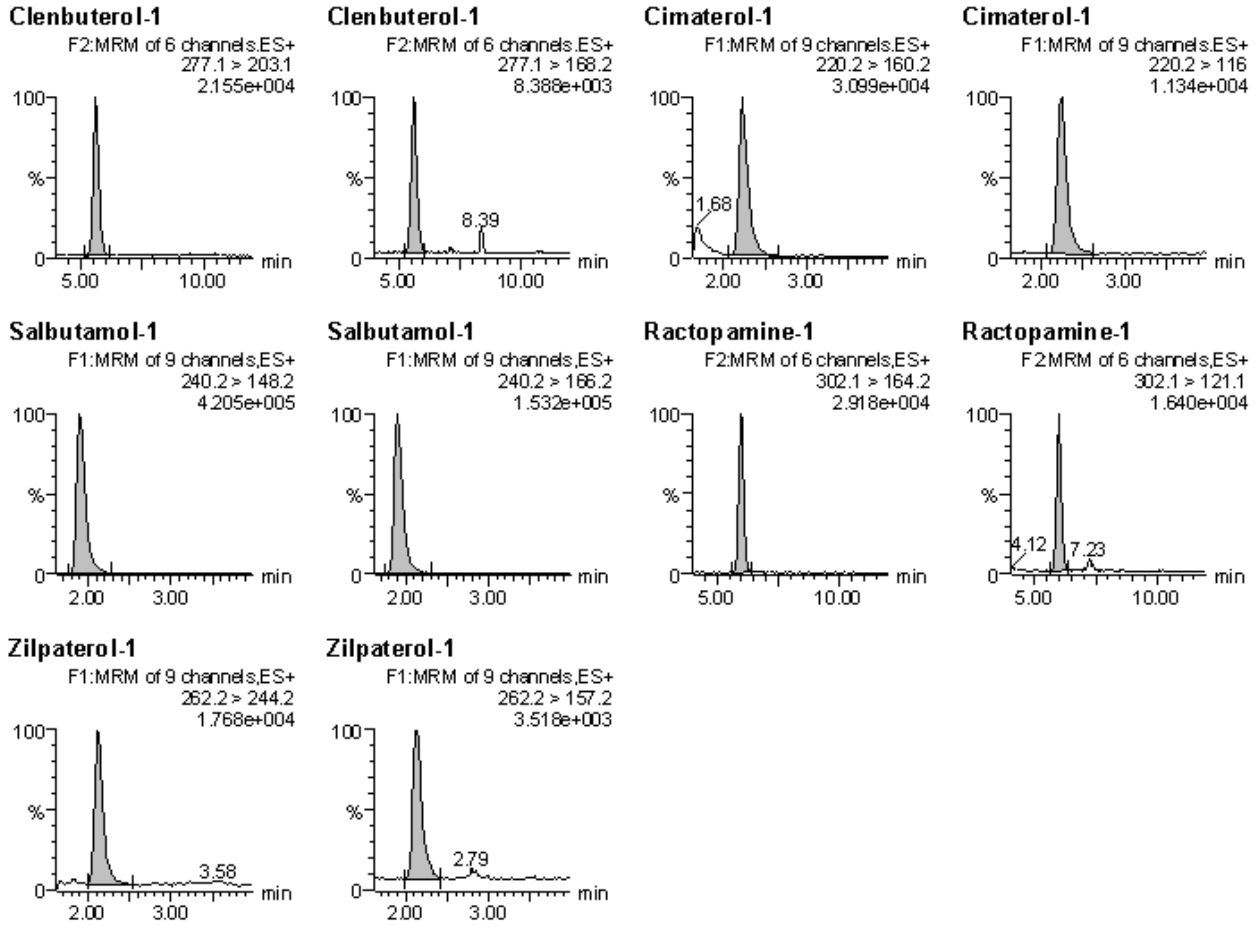
e. Caprine Fortified Liver, 3 ppb



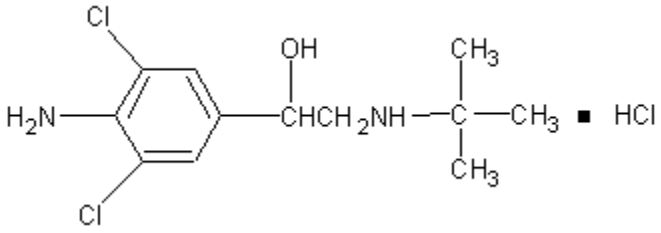
f. Bovine Fortified Muscle, 3 ppb



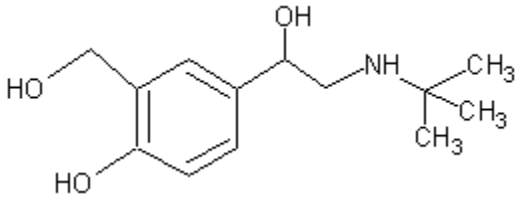
g. Porcine Fortified Muscle, 3 ppb



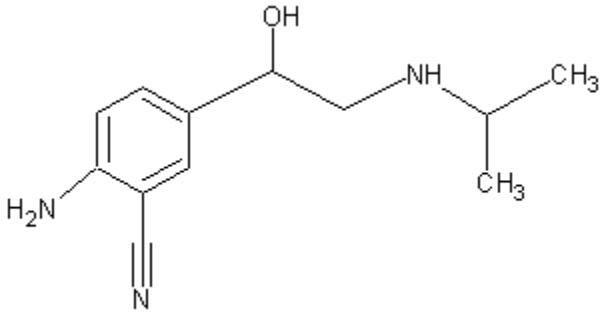
3. Structures



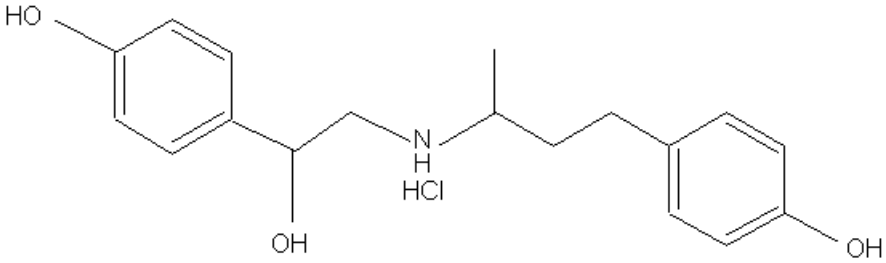
a. Clenbuterol ● HCl



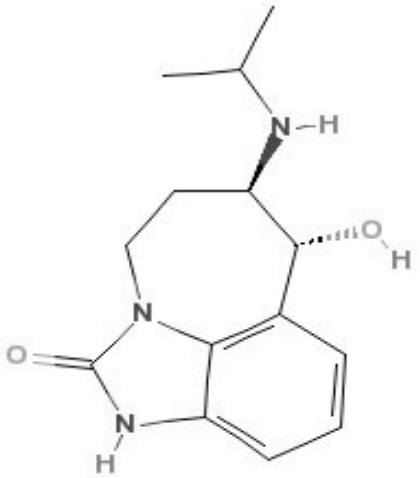
b. Salbutamol



c. Cimaterol



d. Ractopamine ● HCl



e. Zilpaterol

K. APPROVALS AND AUTHORITIES

1. Approvals on file.

2. Issuing Authority: Director, Laboratory Quality Assurance Division.