



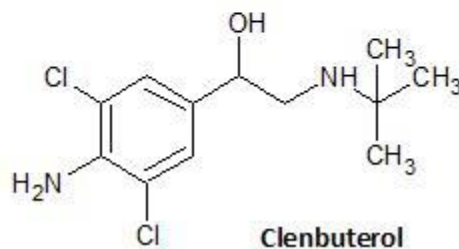
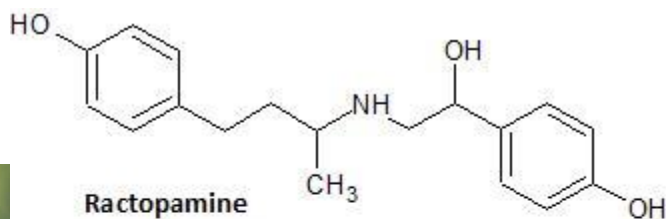
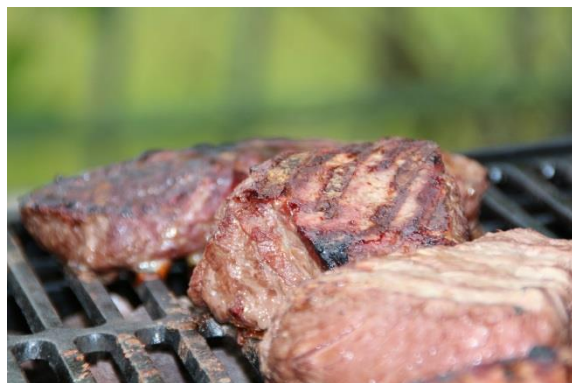
# Determination of Ractopamine and Clenbuterol in Beef Samples

Application Note FB0115

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## Introduction

Ractopamine and clenbuterol are  $\beta$ 2-agonist drugs added to animal feed in order to promote rapid growth of lean muscle in swine, cattle, and turkey feed. Ractopamine, which currently has no approved use in humans, has been banned in around 160 countries, including all of the European Union, China, and Russia. The RR-isomer of ractopamine has been shown to have potent cardiotoxic effects in humans<sup>1</sup>. Clenbuterol, sometimes prescribed as a bronchodilator for the treatment of asthma, has also found off-label use as a weight loss or performance-enhancing drug due to its thermogenic properties. Prolonged exposure to  $\beta$ -agonist drugs can lead to tachycardia, muscle tremor, vasodilation, and adverse metabolic effects<sup>2</sup>. Automated SPE offers unattended sample processing, consistent and reproducible results, and significant operator time savings with excellent sample recovery, even at very low analyte concentrations and established U.S. FDA tolerance limits of 30 ppb<sup>3</sup> for residual ractopamine in cattle muscle.





## Materials & Methods

### Sample Preparation

Add 5 mL of 0.2N sodium acetate (pH 5.2) to 1 g ground meat spiked with 100  $\mu$ L internal standard (250 ng/mL ractopamine-d6 and 250 ng/mL clenbuterol-d9 in MeOH) and homogenize. Add 50  $\mu$ L  $\beta$ -Glucuronidase/Arylsulfatase, vortex for 30 s, and incubate at 37 °C overnight, followed by the addition of 2.5 mL 0.1 M perchloric acid, 2 mL 0.1 M HClO<sub>4</sub>, 2 mL 4% H<sub>3</sub>PO<sub>4</sub> in ACN, and 5 mL 0.5M glycine (pH 10.5). Adjust pH to 10.5 before adding 10 mL ACN and the contents of one SiliaQuick™ QuEChERS Liquid Extraction Salt Packet for the Original Method (PN QE-0001-100P); shake for 30 s and centrifuge at 3000 RPM for 2 min. Transfer organic phase to a tube and extract with an additional 10 mL ACN in a similar manner. Evaporate the combined organic phases from the two extractions to dryness and reconstitute with 4 mL of 0.1 M HClO<sub>4</sub>.

<b>Sample Cleanup- Solid Phase Extraction</b>	
<b>Liquid Handler with Syringe Pumps:</b>	Gilson GX-274 + 406 Dual Syringe Pumps
<b>Cartridge:</b>	SiliaPrepX WCX 3 cc 60 mg (SiliCycle PN: SPE-P0015-03BB)
<b>Condition:</b>	1. 3000 $\mu$ L MeOH at 6 mL/min 2. 3000 $\mu$ L Water at 6 mL/min
<b>Load:</b>	2000 $\mu$ L extract at 5 mL/min
<b>Wash:</b>	1. 1500 $\mu$ L 25 mM phosphate buffer (pH 7) at 6 mL/min 2. 3000 $\mu$ L Water at 6 mL/min 3. 1000 $\mu$ L MeOH at 6 mL/min
<b>Elute:</b>	1. 3000 $\mu$ L 2% formic acid in MeOH at 6 mL/min 2. Evaporate to dryness 3. Reconstitute with 1500 $\mu$ L 65:35 H <sub>2</sub> O:MeOH

<b>LC/MS/MS Detection</b>	
<b>Column:</b>	SiliaChrom dt C18, 2.5 $\mu$ m, 50 x 3.0 mm (SiliCycle PN: H141802E-H050)
<b>Flow rate:</b>	0.600 mL/min
<b>MS Splitting flow rate</b>	0.300 mL/min
<b>Mobile phase:</b>	1 mM ammonium formate in 65:35 Water:MeOH with 0.1% formic acid (v/v)
<b>Temperature:</b>	23°C
<b>Injection volume:</b>	5 $\mu$ L
<b>Detector:</b>	Sciex API 3000
<b>Ionization Mode:</b>	ESI+
<b>Turbo Ion Spray Heater Gas Flow:</b>	8,000 cc/min
<b>Turbo Ion Spray Heater Temperature:</b>	400°C



Reference NRM Transitions:	
Clenbuterol	277.0 -> 203.0
Clenbuterol d-9 (IS)	286.2 -> 204.1
Ractopamine	302.2 -> 164.2
Ractopamine d-6 (IS)	308.2 -> 168.2

## Results and Discussion

Ractopamine and clenbuterol were isolated from beef samples using the Gilson GX-274 ASPEC system. Both analytes yielded recovery values of greater than 89% across the concentration range tested (3-70 ppb; see Table 2).

Quantification was performed using ESI+ mass spectrometry. Calibration curves with R<sup>2</sup> values of 0.9968 and 0.9978 for ractopamine and clenbuterol, respectively, were generated prior to sample quantitation by mass spectrometry.

**Table 1.** Detection and quantification limits for clenbuterol and ractopamine

	Ractopamine	Clenbuterol
Limit of Detection (ppb)	0.027	0.135
Limit of Quantification (ppb)	0.045	0.230
Ionic Suppression (%)	0	9

Ppb= parts per billion

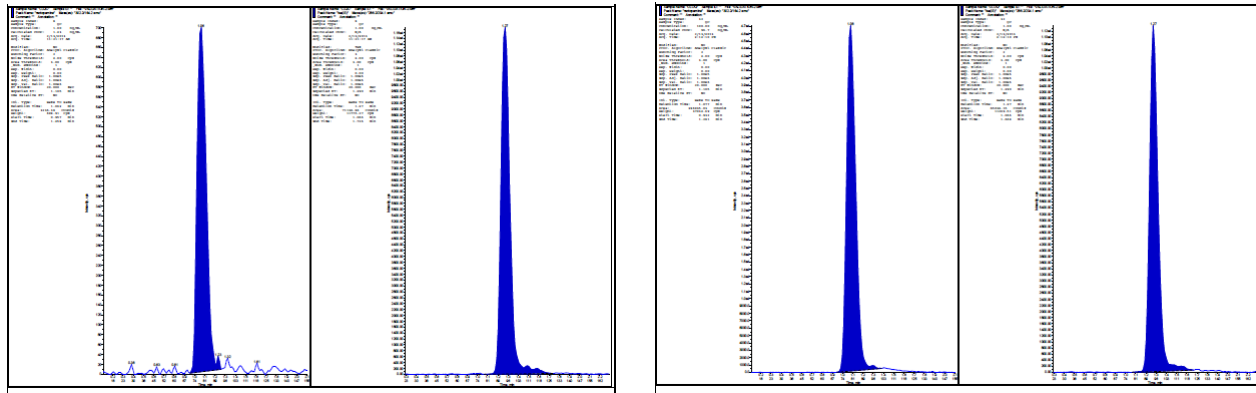
**Table 2.** Accuracy and recovery for clenbuterol and ractopamine over range of concentration levels.

Concentration Level	Concentration (ppb)	Clenbuterol		Ractopamine	
		Accuracy (%)	Recovery (%)	Accuracy (%)	Recovery (%)
LLQC	1.0	97 ± 1	N/A	104 ± 1	N/A
3X LLQC	3.0	99 ± 6	95 ± 4	102 ± 4	90 ± 2
30% ULQC	30.0	105 ± 2	93 ± 2	103 ± 3	89 ± 2
70% ULQC	70.0	105 ± 3	92 ± 1	106 ± 4	91 ± 2
ULQC	100.0	99 ± 6	N/A	100 ± 3	N/A

LLQC = Lower Limit of Quantification Control; ULQC = Upper Limit of Quantification Control

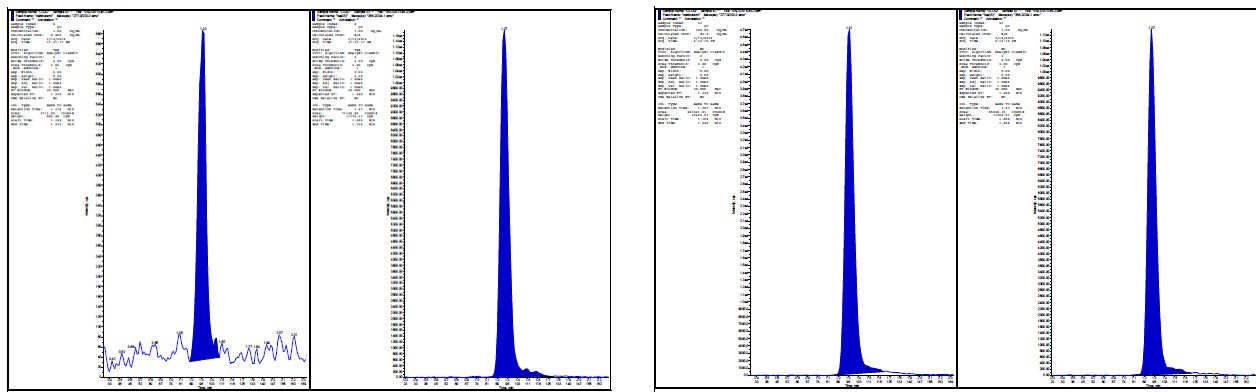


## Ractopamine



**Figure 1.** Mass spectra for ractopamine at the lower (left) and upper (right) levels of quantification control.

## Clenbuterol



**Figure 2.** Mass spectra for clenbuterol at the lower (left) and upper (right) levels of quantification control.

## Summary

- The Gilson GX-274 ASPEC system provides an automated solution for unattended, reproducible isolation of clenbuterol and ractopamine, two common controversial cattle feed additives, from muscle samples.
- Ractopamine and clenbuterol were successfully isolated from beef samples with recoveries comparable to literature values<sup>4</sup>, even with analyte concentrations lower than the residual limit established by the U.S. FDA.
- Meat samples can be reliably and efficiently cleaned up for ractopamine and clenbuterol quantitation with the Gilson GX-274 ASPEC system.



## References

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3. U.S. FDA; CFR - Code of Federal Regulations Title 21, Part 556 – Tolerances for Residues of New Animal Drugs in Food; Subpart B – Specific Tolerances for Residues of New Animal Drugs; Sec. 556.570 - Ractopamine. [Online] <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=556.570>
4. J Chromatogr Sci. 2009 Apr;47(4):324-8.