

Rapid and Sensitive Determination of Clenbuterol, Ractopamine, and Phenoethanolamine in Meat

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Key Words

β_2 -Agonists, Dionex SolEx SPE Cartridge, Acclaim PolarAdvantage (PA) Column, On-Line Solid-Phase Extraction (SPE), High-Performance Liquid Chromatography (HPLC), Food Analysis

Goal

To develop an efficient HPLC method for the rapid and sensitive determination of clenbuterol, ractopamine, and phenoethanolamine in meat using on-line SPE and UV detection with method detection limits (MDL) meeting those obtained by mass spectrometry (MS)

Introduction

Clenbuterol, ractopamine, and phenoethanolamine (structures shown in Figure 1) are β_2 -agonists that can stimulate the nervous system. They have been extensively used as growth stimulants in farm animals and by athletes seeking enhanced performance. Abuse of these substances can cause side effects in humans (e.g., increased heart rate and blood pressure, anxiety, palpitation, and skeletal muscle tremors). Therefore, clenbuterol—the most effective β_2 -agonist used as a growth-promoting agent for farm animals—has been banned as a food/feed additive in many countries, including the USA, European Union (EU), and People's Republic of China. Ractopamine was commercially developed in the USA, where its use is authorized; however, in the EU and People's Republic of China, the use of ractopamine is completely banned. Phenoethanolamine—recently introduced as a new β_2 -agonist—already has been banned in the People's Republic of China.



Liquid chromatography-MS and gas chromatography-MS are typically used for sensitive determination of clenbuterol and ractopamine. SPE is the typical method used for sample extraction and enrichment prior to chromatography.¹⁻⁴ Compared to off-line SPE, on-line SPE offers the advantages of full automation, the absence of operator influence, time savings, and strict process control. The work shown here provides a sensitive and rapid HPLC method with on-line SPE and UV detection for simultaneous determination of clenbuterol, ractopamine, and phenoethanolamine in meat.

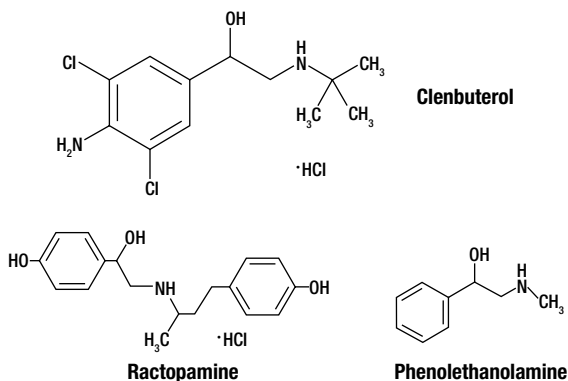


Figure 1. Structures of clenbuterol, ractopamine, and phenoethanolamine.

Equipment, Software, and Consumables

- Thermo Scientific™ Dionex™ UltiMate™ 3000 Dual RSLC system, including:
 - DGP-3600RS Dual Gradient Pump (P/N 5040.0066)
 - SRD-3600 Integrated Solvent and Degasser Rack (P/N 5035.9230)
 - WPS-3000TRS Wellplate Sampler, Thermostatted (P/N 5840.0020), with a 2500 µL Sample Loop and a 2500 µL Syringe
 - TCC-3000RS Thermostatted Column Compartment (P/N 5730.0000)
 - DAD-3000RS Diode Array Detector, without Flow Cell (P/N 5082.0020)
 - Semi-Micro Flow Cell for DAD-3000 and MWD-3000 Series, SST, 2.5 µL Volume, 7 mm Path Length (P/N 6082.0300)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software, version 7.1 or higher
- Thermo Scientific™ Orion™ 2-Star Benchtop pH meter
- Thermo Scientific™ Target2™ Nylon Syringe Filters, 0.45 µm, 30 mm (P/N F2500-1)

Conditions

On-Line SPE

Cartridge:	Thermo Scientific™ Dionex™ SolEx™ SPE, 2.1 × 20 mm (P/N 074400), with V-3 Cartridge Holder (P/N 074403)
Mobile Phase:	A. 1.0 mM Na ₂ HPO ₄ /4.0 mM NaH ₂ PO ₄ /0.1 mM Na ₄ P ₂ O ₇ (pH 6, dissolve 0.178 g of Na ₂ HPO ₄ ·2 H ₂ O, 0.624 g of NaH ₂ PO ₄ ·2 H ₂ O, and 0.266 g of Na ₄ P ₂ O ₇ in 1 L deionized [DI] water, without pH adjustment) B. CH ₃ CN
Gradient:	0–3 min, 0% B; 3–6.6 min, 0–50% B; 6.6–8 min, 50% B; 8.1–10 min, 0% B
Flow Rate:	0.4 mL/min
Injection Volume:	2500 µL on the on-line SPE cartridge

Separation

Column:	Thermo Scientific™ Acclaim™ PolarAdvantage, 3 µm Analytical, 3.0 × 150 mm (P/N 063693)
Mobile Phase:	A. 12.5 mM HCOONH ₄ /50 mM HCOOH (pH 3, dissolve 0.788 g of HCOONH ₄ and 1.87 mL of HCOOH [99%] in 1 L DI water, without pH adjustment) B. CH ₃ CN C. H ₂ O
Gradient:	0–3 min, 20% A, 5% B; 0–3 min, 20% A, 5% B; 3–6.5 min, 20% A, 5–65% B; 6.5–8 min, 20% A, 65% B; 8.1–10 min, 20% A, 5% B
Flow Rate:	0.6 mL/min
Temperature:	25 °C
Detection:	UV, absorbance at 210 nm
Valve Position:	0 min, 1_2; 2.9 min, 6_1; 8 min, 1_2

Sample Preparation

Add 8 mL of DI water to a 2 g sample of mashed pork in a 15 mL centrifuge tube; after 20 min of vortex mixing, followed by 30 min in an ultrasonic bath, add 2 mL dilute acetic acid (3%, v/v); then store the solution at 4 °C for at least 1 h. After 5 min of centrifugation (rpm 10000) transfer the supernatant to a 10 mL volumetric flask and add water to the mark. Prior to injection, filter the solution through a 0.45 µm filter.

Results and Discussion

To develop the on-line SPE method, it was necessary to determine the fidelity of retention of clenbuterol, ractopamine, and phenoethanolamine on the SPE cartridge and evaluate their ease of elution from the cartridge. The Dionex SolEx SPE cartridge, packed with a divinylbenzene polymer with a hydrophilic bonded layer, has already been applied to the on-line SPE HPLC analysis of carbamates, aniline, and nitroanilines.^{5–7} The Dionex SolEx SPE cartridge was selected as the SPE column due to its excellent retention properties for clenbuterol, ractopamine, and phenoethanolamine.

Figure 2, Chromatogram A illustrates good separation of clenbuterol, ractopamine, and phenoethanolamine following on-line SPE under the specified chromatographic conditions. Method precision was estimated using UV detection by making seven consecutive 2500 μL injections of a standard mixture with a concentration of 10 $\mu\text{g}/\text{L}$ of each compound. The retention time and peak area reproducibilities are summarized in Table 1 and show good precision. The method MDLs for UV detection—calculated using a signal-to-noise ratio = 3—were 1 $\mu\text{g}/\text{L}$ for clenbuterol, 0.15 $\mu\text{g}/\text{L}$ for ractopamine, and 0.5 $\mu\text{g}/\text{L}$ for phenoethanolamine, which are equivalent to those obtained by MS detection.¹⁻⁴ This demonstrates that these three β_2 -antagonists can be efficiently captured, eluted, and separated with the described method. Clenbuterol, with an estimated concentration of 10 $\mu\text{g}/\text{L}$, was found in the pork sample (Figure 2, Chromatogram B), demonstrating that this pork was contaminated and not legal for sale to a consumer.

For determination of clenbuterol, ractopamine, and phenoethanolamine in meat samples (e.g., pork products), the cleanup procedure before on-line SPE is critical. Several reported cleanup procedures were tried. The estimated recovery of clenbuterol was in the range of 50~60%, whereas the sample preparation shown in this work yielded an ~75% recovery of clenbuterol.¹⁻⁴ No procedure was found that yielded adequate recovery for ractopamine and phenoethanolamine in pork.

Conclusion

This work describes an on-line SPE HPLC method with UV detection for rapid and sensitive determination of clenbuterol, ractopamine, and phenoethanolamine in meat products. The MDLs for UV detection are ≤ 1 $\mu\text{g}/\text{L}$, which are equivalent to those obtained by MS detection. The determination was performed using an UltiMate 3000 x2 Dual RSLC system controlled by Chromeleon CDS software.

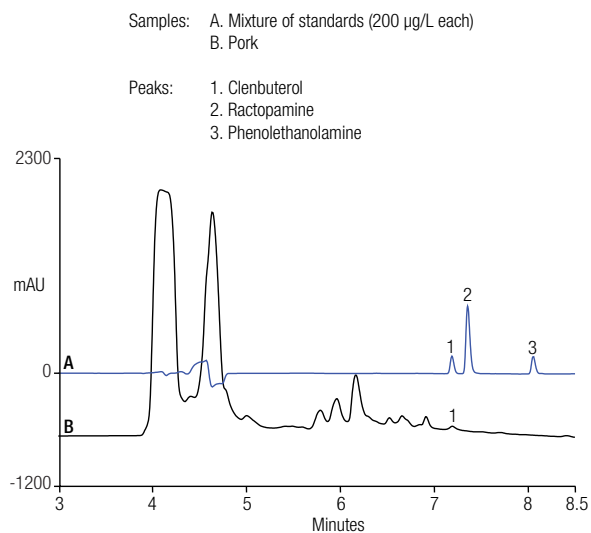


Figure 2. (A) A mixture of standards and (B) a pork sample.

Table 1. Reproducibility of retention time and peak area.

Analyte	Retention Time RSD	Peak Area RSD
Clenbuterol	0.04	2.53
Ractopamine	0.04	1.99
Phenoethanolamine	0.04	2.33

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