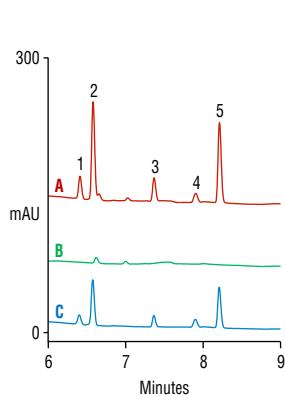


Beta-agonists in Pork Using In-line SPE



Analytical Column: Thermo Scientific™ Acclaim™ C30 3 μ m
 Dimension: 2.1 \times 150 mm
 SPE Column: Thermo Scientific Acclaim Mixed-Mode WCX-1 guard cartridge
 Dimension: 3.0 \times 10 mm
 HPLC System: Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system x2 dual gradient
 Mobile Phase: Left pump A: Methanol
 Left pump B: 5 mM ammonium formate, pH 7.5
 Right pump A: Acetonitrile
 Right pump B: Water
 Right pump C: 12.5 mM ammonium formate, 50 mM formic acid, pH 3.1
 Gradient Times (min): -3.0 0.0 0.5 0.5 3.0 4.6 8.0 9.0 9.0 12.0 12.1 15.0
 Left pump %A: 100 100 100 50 50 100
 Left pump %B: 0 0 100 50 50 100
 Right pump %A: 5 5 5 65 65 5 5
 Right pump %B: 75 75 75 17 17 75 75
 Right pump %C: 20 20 20 18 18 20 20
 Aux. valve: load inject load
 Flow Rate: Right: 0.40 mL/min, left: 0.40 mL/min
 Injection: 100 μ L
 Aux. Valve: 10-port, 2-position configured for reverse elution of SPE
 Temperature: 25 °C
 Detection: Diode Array, UV at 210 nm, 2 Hz, 0.5 s resp. time
 Peaks:
 1. Salbutamol
 2. Cimaterol
 3. Ritodrine
 4. Ractopamine
 5. Clenbuterol

Samples:
 A. Pork chop extract + standards at 200 μ g/kg
 B. Pork chop extract
 C. Standards at 100 μ g/L
 Sample preparation:
 Extract 5.0 g pork chop with approx. 50 mL of 4:1 acetonitrile:isopropanol using ASE extractor.
 Chill to precipitate fat, and filter.

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Clenbuterol is the most well-publicized member of a group of beta-adrenergic agonist and non-steroidal anabolic agents that decrease body fat and increase muscle. Unethical farmers and athletes are known to use these drugs. There have been public outbreaks of clenbuterol poisoning due to contaminated meat. The U.S.

Department of Agriculture tests animal carcasses for a handful of these drugs (http://www.fsis.usda.gov/PDF/CLG_AGON_1_03.pdf). The World Anti-Doping Agency has banned these drugs, and routinely tests athletes for them. Since trace levels are very low, some form of cleanup and concentration is required.

In this example, we extracted pork with organic solvents in an Accelerated Solvent Extractor. Evaporation and reconstitution proved troublesome, so we avoided that by using solid-phase extraction (SPE). SPE is most effective when it is orthogonal to the analytical separation, so we used cation exchange to retain the drugs, and washed away lipids and other interferences with organic solvent. The auxiliary valve then transferred the drugs to an Acclaim C30 column for RP-HPLC analysis.