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Introduction

- > The β2-agonists have been used worldwide as illegal growth promoters in pork production.
- Recently incidences of poisoning occurred due to high levels of the β-agonist (clenbuterol) in pork.
- Solid-Phase Extraction is an efficiency sample preparation method for extraction and purification of β2-agonists in pork.
- > This application note used Agilent's new SCX polymer cartridges to extract and enrich four β-agonists from pork and analysis by LC-MS/MS.

β2-Agonist Compounds Used in this Study

Compound	LogP	Structure
Terbutaline	0.55	HO OH H
Salbutamol	0.44	HO OH H
Clebuterol	2.94	CI H ₂ N CI
Formoterol	1.91	OH H NOOH

Masses Monitored in the MRM

Compund	MRM for Quantification	MRM for Confirmation
Salbutamol	240 → 148	240 → 222
Terbutaline	226 → 152	226 → 125
Clenbuterol	277 → 203	277 → 259
Formoterol	345 → 149	345 → 327



Experimental

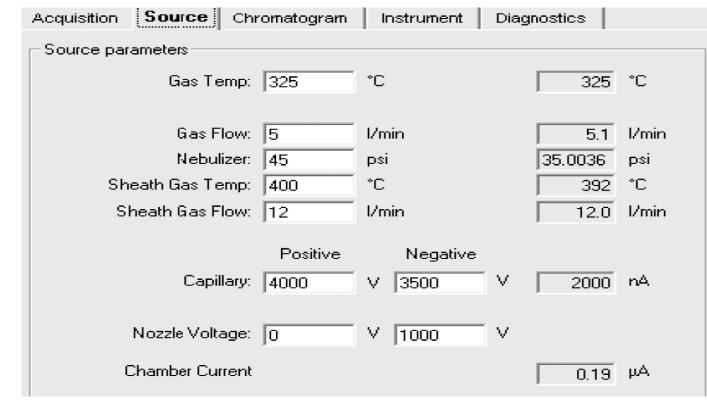
Instrument Conditions

Analytical column: Zorbax Eclipse Plus C18 2.1×50mm 1.8μm

Flow rate: 0.4 mL/min

Mobile phase: A. 0.1% formic acid and 2 mmol/L ammonium acetate B. acetonitrile

Gradient: Ti	me (min) A%	В%	
	. 0	90	10
	0.5	90	10
	1.8	20	80
	2	90	10
	3.5	90	10





Sample preparation: liquid-liquid extraction

Accurately weigh 2 g comminuted sample in 15 mL centrifuge tube

Add 8 mL 0.2 M sodium acetate solution and 100 μL βglucuronidase (1000 U/mL), vortex thoroughly

Hydrolyze at 37 °C for 16 hours

Centrifuge at 4000 rpm for 10 minutes

Hydrolyze at 37 °C for 16 hours

Transfer 4 mL amount of supernatant to another centrifuge tube

Add 5 mL 0.1 M perchloric acid solution and adjust pH to 1 \pm 0.3

Centrifuge at 4000 rpm for 10 minutes

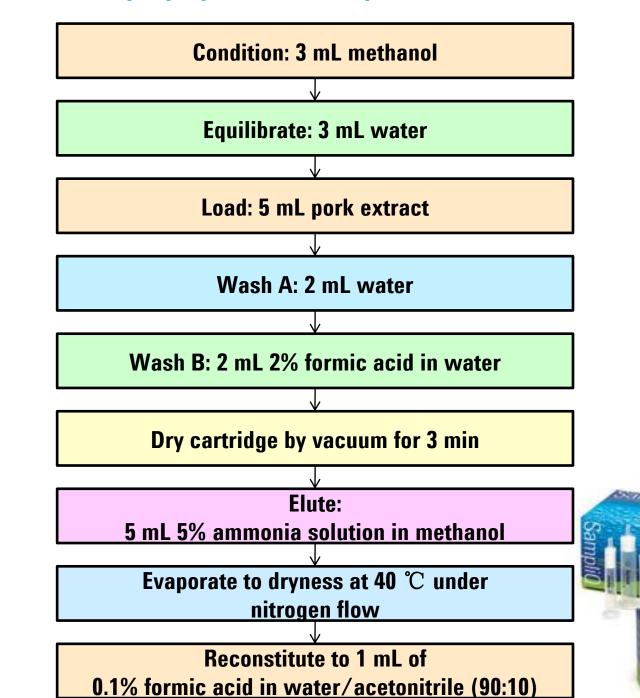
Transfer supernatant to another tube and adjust pH to 11 with 10 M sodium hydroxide

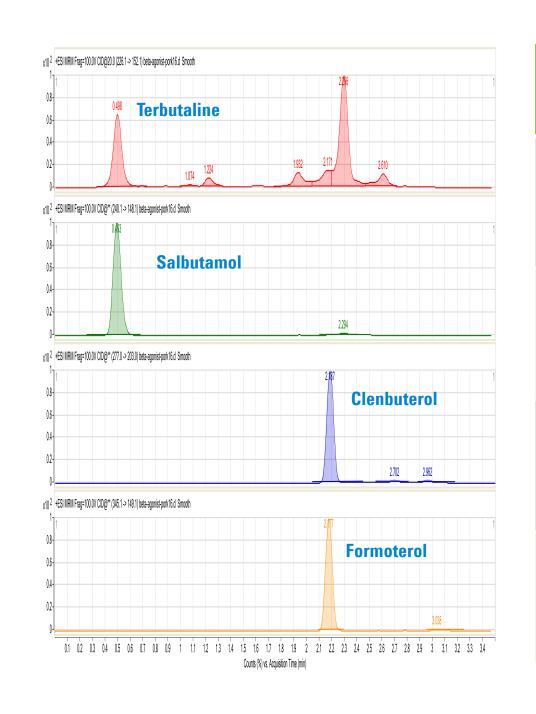
Add saturated sodium chloride solution isopropanol-ethyl acetate (60:40) and extract twice, combine all supernatant

Evaporate to dryness with nitrogen at 40 ° C. Dissolve residue in 5 mL of 0.2 M sodium acetate (pH 5.2)

Experimental

Sample preparation: solid-phase extraction





Compund	Spiked level (ng/g pork)	Recovery (%)	RSD (n=6)
Terbutaline	0.5	88.7	5.4
	1	98.0	7.2
	2	100.8	5.9
Salbutamol	0.5	100.6	1.8
	1	92.9	2.1
	2	97.4	3.9
Clenbuterol	0.5	82.3	5.0
	1	91.5	6.3
	2	90.6	4.3
	0.5	85.1	1.9
Formoterol	1	83.0	4.0
	2	77.9	2.5

