

β₂-Agonists in Pork and Pig Liver Tissues

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates β₂-Agonists in pork and pig liver tissues.

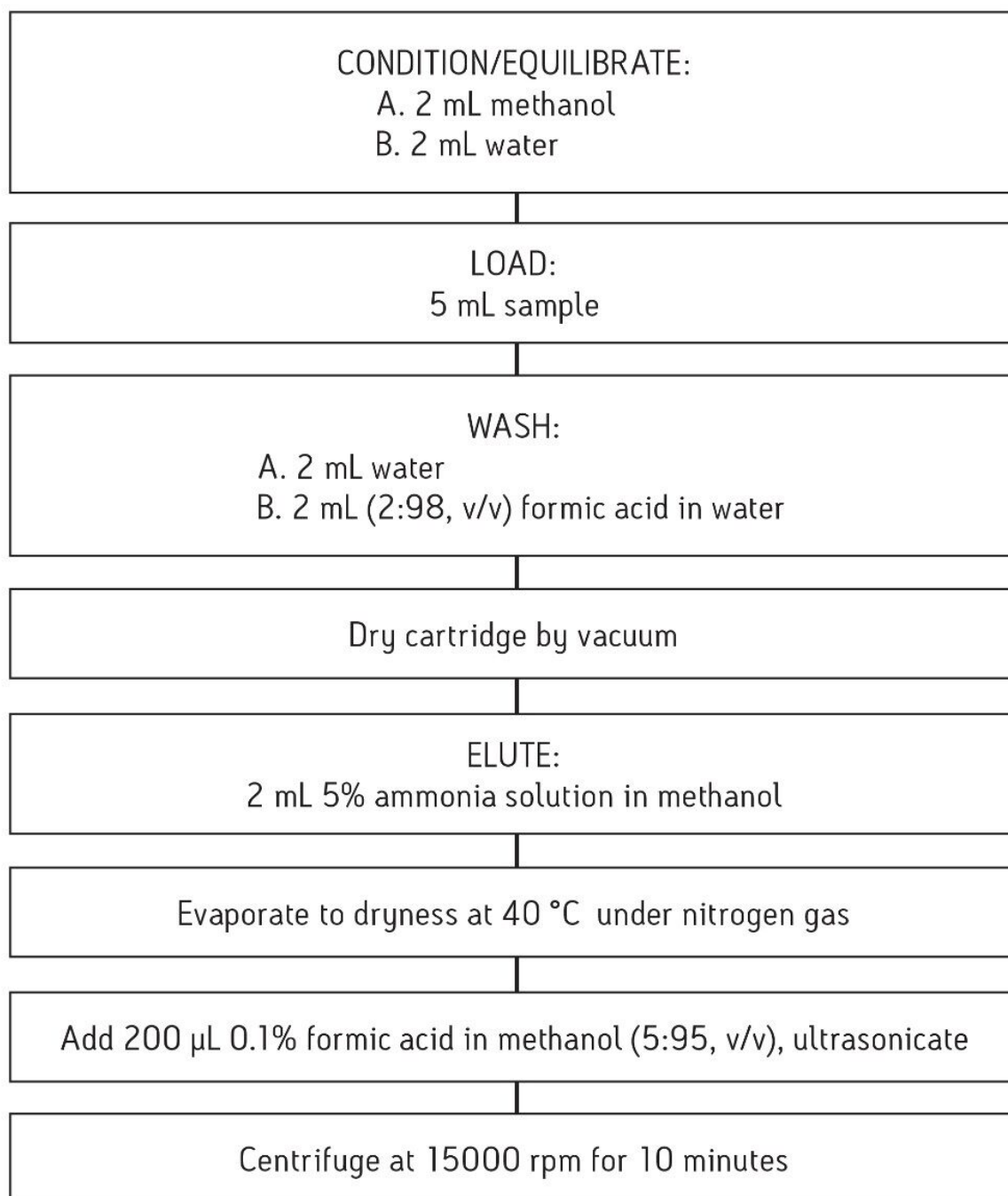
Experimental

Pretreatment

1. Add 8 mL 0.2 M sodium acetate (pH 5.2) to 2 g of sample. Homogenize and take out supernatant. Add 50 μ L β -Glucuronidase/arylsulfatase and hydrolyze at 37 °C overnight.
2. Shake the hydrolysate for 15 minutes. Centrifuge at 5000 rpm for 10 minutes and take out 4 mL supernatant.
3. Add 100 μ L of 10 ng/mL standards (clenbuterol-D₉, salbutamol-D₃) and mix.
4. Add 5 mL 0.1 M perchloric acid and adjust pH to 1 ± 0.3 .
5. Centrifuge at 5000 rpm for 10 minutes.
6. Collect supernatant and add 10 M sodium hydroxide to adjust pH to 11.
7. Add 10 mL saturated sodium chloride and 10 mL isopropanol-ethyl acetate (60:40, v/v).
8. After centrifugation, take organic layer and evaporate to dryness at 40 °C under nitrogen gas.
9. Dissolve residue in 5 mL 0.2 M sodium acetate (pH 5.2).

SPE Procedure

Oasis® MCX 3 cc/60 mg



LC Conditions

Instrument:	Waters Alliance HPLC 2695 System
Analytical column:	Atlantis dC ₁₈ , 2.1 x 150 mm, 5 µm
Guard column:	Atlantis dC ₁₈ , 2.1 x 10 mm, 5 µm
Flow rate:	0.2 mL/min
Mobile phase:	A. 0.1% formic acid B. 0.1% formic acid in acetonitrile
Injection volume:	20 µL
Column temperature:	35 °C

Gradient

Time (min)	A%	B%
0	96	4
2	96	4
8	77	23
21	77	23
22	5	95
25	5	95
25.5	96	4

MS Conditions

Instrument: Waters Quattro Premier

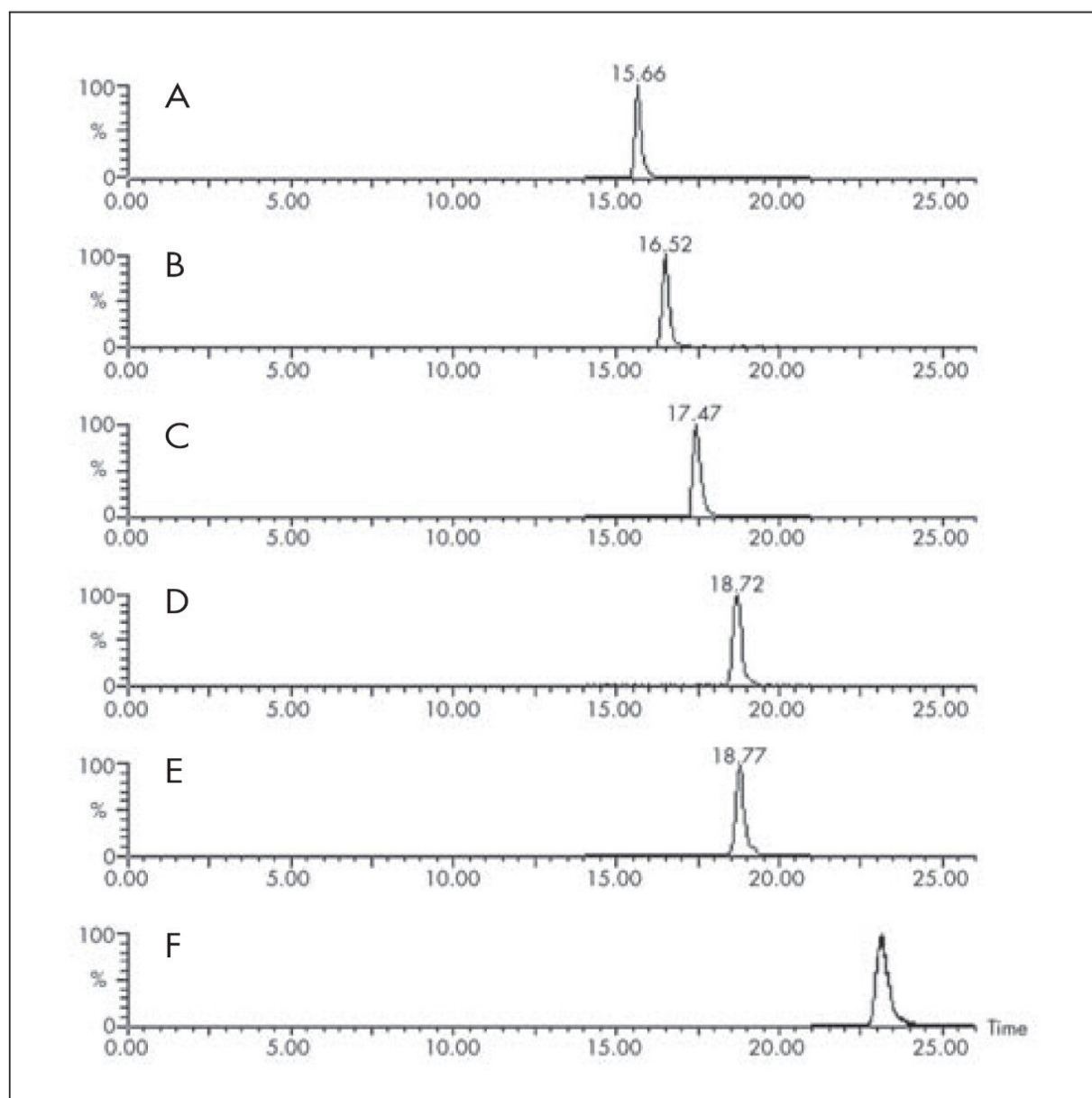
Ionization mode: Positive electrospray (ESI⁺)

Multiple reaction monitoring

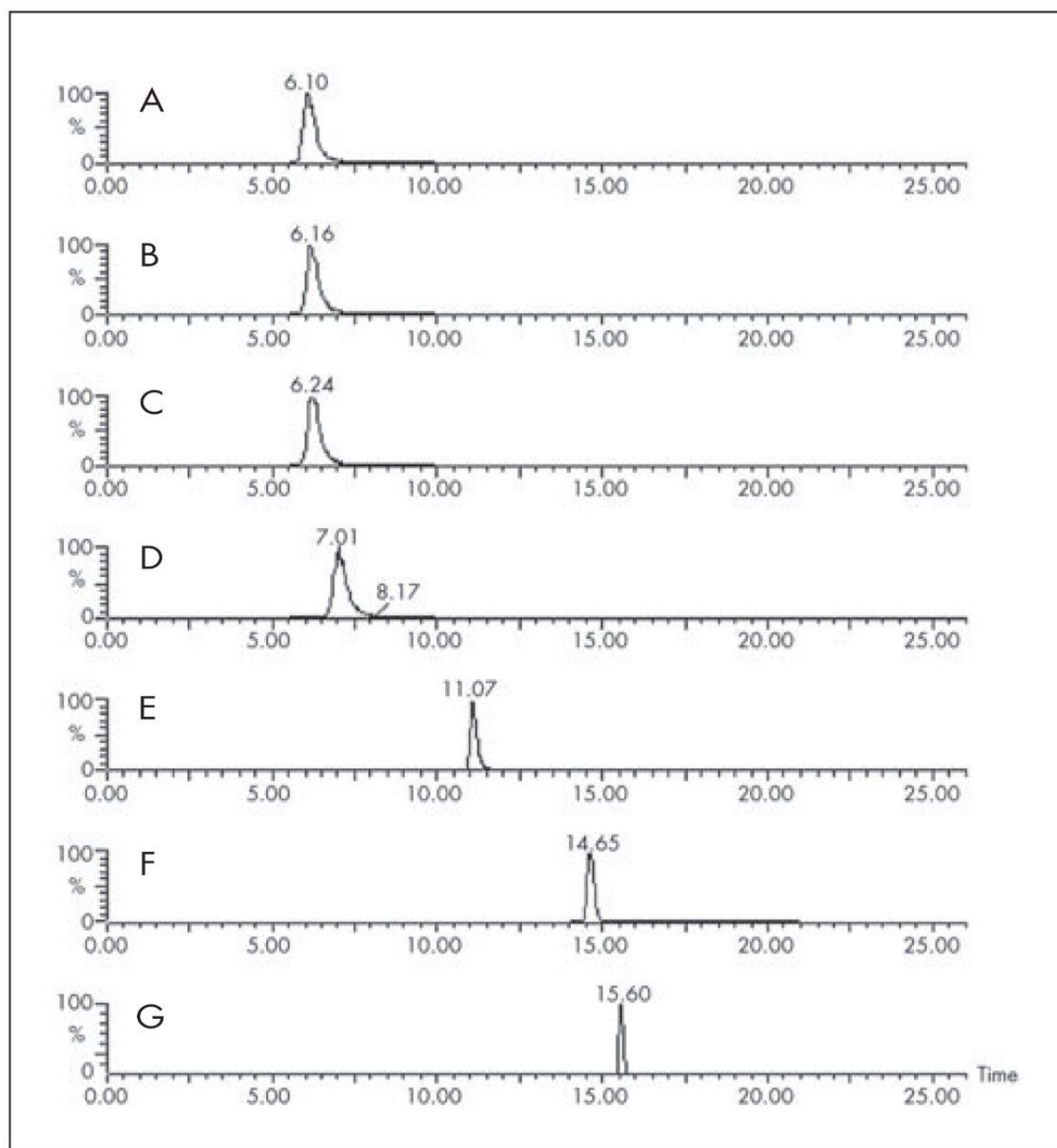
Analyte	MRM for Quantification	MRM for Confirmation
Salbutamol	240 → 148	240 → 222
Terbutaline	226 → 152	226 → 125
Cimaterol	202 → 160	202 → 143
Cimbuterol	234 → 160	234 → 143
Ractompamine	302 → 164	302 → 284
Clenbuterol	277 → 203	277 → 259
Bromclenbuterol	323 → 249	323 → 168
Bromobuterol	367 → 293	367 → 349
Isoxsuprine	302 → 150	302 → 284
Mabuterol	311 → 237	311 → 293
Mapenterol	325 → 237	325 → 217
Clenbuterol-D ₉ (IS)	286 → 204	286 → 204
Salbutamol-D ₃ (IS)	243 → 151	243 → 151

MRM method parameters.

Results and Discussion



Chromatograms of 6 β_2 -agonists by multiple reaction monitoring (MRM) scan mode (A) clenbuterol (B) bromclenbuterol (C) bromobuterol (D) isoxxsuprine (E) mabuterol (F) mapenterol.



Chromatograms of 7 β_2 -agonists by multiple reaction monitoring (MRM) scan mode (A) salbutamol- d_3 (B) salbutamol (C) terbutaline (D) cimaterol (E) cimbuterol (F) ractompamine (G) clenbuterol- D_9 .

Pig liver tissues were spiked with 11 β_2 -agonists standard mixture of concentrations 0.5 ng/g, 1 ng/g and 2 ng/g respectively. The SPE recoveries are between 89.4% and 110.5%, RSD are between 1% and 2.8%.

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Alliance HPLC System <<https://www.waters.com/534293>>

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