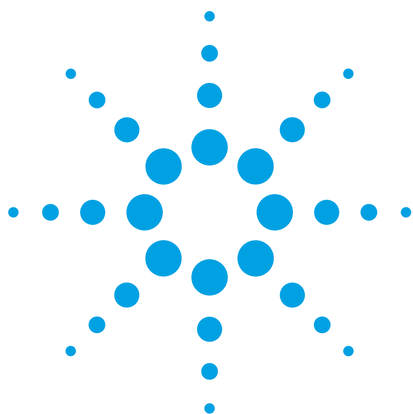

Determination of Dapsone in Bovine Muscle with Agilent Bond Elut SPE and LC/MS/MS

Application Note

Food Testing and Agriculture

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Introduction

In this application note, bovine muscle was prepared and analyzed for dapsone at the sub-ppb level, using a straightforward SPE method with Agilent Bond Elut Plexa PCX SPE, efficient separation with an Agilent Poroshell 120 LC column, and sensitive detection with an Agilent 6460 Triple Quadrupole LC/MS system.

Materials and Methods

HPLC conditions	
Column:	Agilent Poroshell 120 SB-C18, 2.1 × 100 mm, 2.7 µm (p/n 685775-902)
Eluent:	A) 0.1% formic acid in water, B) acetonitrile
Injection volume:	10 µL
Flow rate:	0.3 mL/min
Gradient:	15 % B, linear to 80 % B in 8 min
Temperature:	Ambient
Sample vials:	Agilent Certified Vials (p/n 5183-2072)
System:	Agilent 1260 Infinity LC system

MS conditions	
Ionization mode:	ESI + Agilent Jet Stream
Gas temperature:	325 °C
Gas flow:	10 L/min
Nebulizer:	50 psi
Sheath temperature:	400 °C
Sheath gas flow:	12 L/min
Capillary:	4,500 V (ESI+)
Nozzle voltage:	0 V (ESI+)
System:	Agilent 6460 Triple Quadrupole LC/MS System

The MRM transitions, fragmentors, and collision energies optimized for dapsone in this study are shown in Table 1.

Table 1. MRM transitions and other conditions for dapsone.

Compound	Precursor ion	Product ion	Fragmentor	CE	Polarity
Dapsone	249.1	92	125	24	Positive
	249.1	108	125	20	Positive

Sample preparation

To pretreat the sample, 2 g of ground bovine muscle was placed into a 50-mL centrifuge tube, followed by 10 mL of acetonitrile. The tube was then vortexed vigorously for 2 minutes. Hexane (5 mL) was placed into the tube and vortexed for 1 minute. Next, the tube was centrifuged for 5 minutes at 5,000 rpm and 4 °C. All of the supernatant was discarded. The acetonitrile layer was transferred into another tube, and 10 mL of 1 M HCl in water was added. The tube was then vortexed for 1 minute, and was ready for SPE.

The procedure shown in Figure 1 was used for sample extraction. Agilent Bond Elut Plexa PCX cartridges (60 mg, 3 mL, p/n 12108603) were preconditioned with 3 mL of methanol followed by 3 mL of water. The extract (equivalent to 2 g of sample) was passed through the cartridge at a rate of 1 mL/min. After the sample passed through completely, the cartridge was washed with 5 mL of 0.1 mol/L HCl in water and 5 mL of methanol. The cartridge was dried under negative pressure (below 2.0 kPa) for 5 minutes. The sample was eluted with 5 mL of 25 % ammonium hydroxide in acetonitrile. The eluate was collected and dried under nitrogen below 40 °C. The sample residue was then dissolved and brought to a constant volume of 1.0 mL using 15 % acetonitrile in water (v:v), filtered through a 0.2-µm filter membrane (Agilent Captiva Polyethersulfone, p/n 5190-5096), and analyzed by LC/MS/MS.

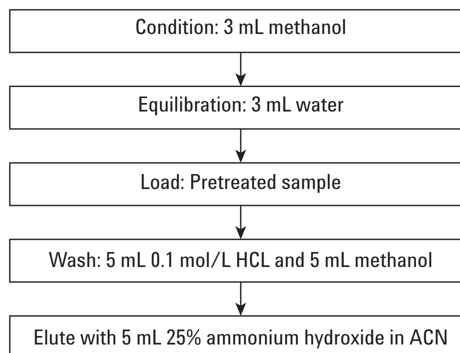

Figure 1. The SPE procedure for a bovine muscle sample.

Results and Discussion

Linearity and limit of detection

Solutions for external calibration curves were prepared by using a combined working solution to spike a matrix blank (0.25, 0.5, 1, 2, and 5 µg/kg). Matrix blanks were created by taking bovine muscle through the entire procedure, including pretreatment and SPE procedures. The limits of detection (LOD) were chosen as the concentration of each compound that gave a signal-to-noise ratio greater than 3:1. The results for the calibration curve and LOD are shown in Table 2.

Table 2. Linearity and LOD of dapsone in bovine muscle.

Compound	Regression equation	R^2	LOD in bovine muscle (µg/kg)
Dapsone	$y = 5985.61x - 3.53$	0.9998	0.02

Recovery and reproducibility

Recovery was measured for dapsone at low and high concentration levels (Table 3). Recovery was calculated by comparing the MRM peak area for samples spiked prior to SPE extraction with the MRM peak area for samples spiked after SPE extraction (post-spiked samples). Figure 2 shows a chromatogram obtained from the analysis of bovine blank sample spiked with a low level of dapsone. Figure 3 is the sample blank.

Table 3. Extraction recovery of dapsone from bovine muscle with SPE.

Compound	Recovery (RSD) n = 6, 0.5 µg/kg	Recovery (RSD) n = 6, 1 µg/kg	Recovery (RSD) n = 6, 2 µg/kg
Dapsone	88.9 (9.7)	96.5 (5.7)	92.8 (3.8)

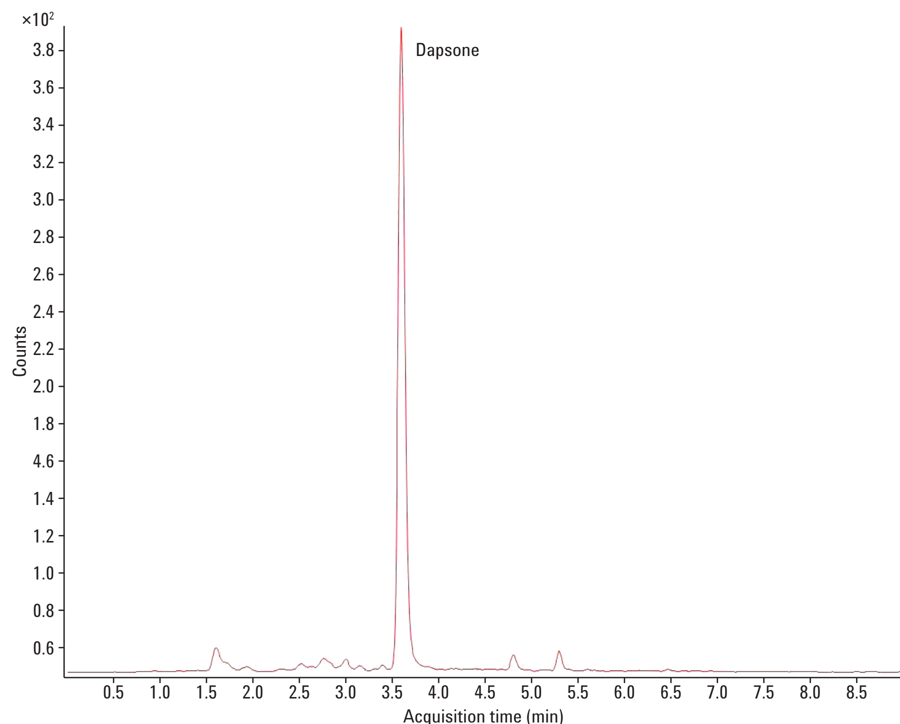

Figure 2. Chromatogram of dapsone obtained from bovine muscle spiked with 1 µg/kg.

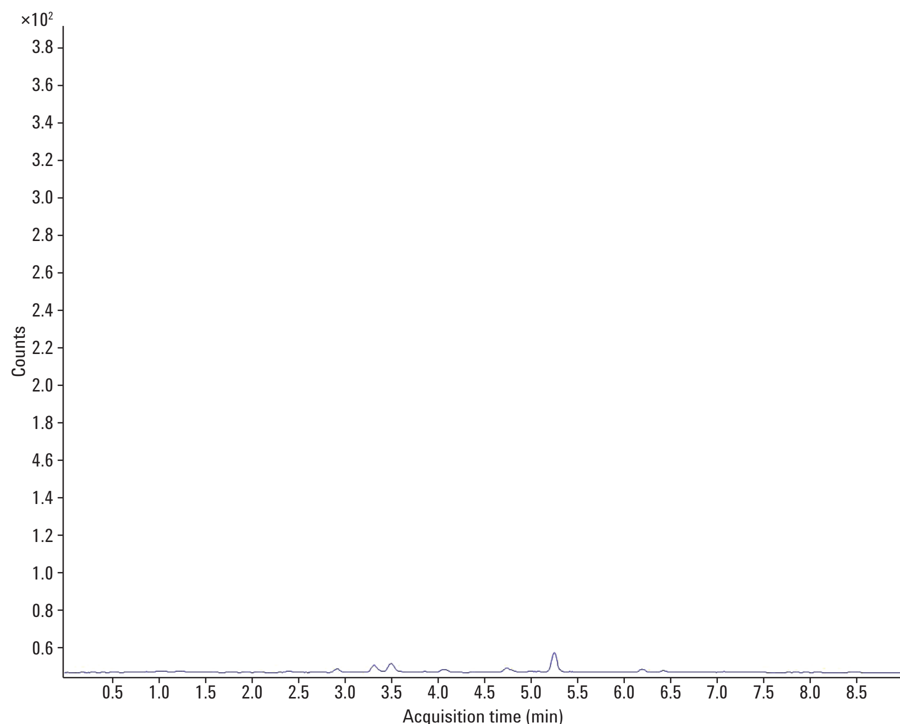

Figure 3. Chromatogram of dapsone obtained from a bovine muscle blank sample.

Conclusions

Good recovery and reproducibility were obtained with Agilent Bond Elut Plexa PCX SPE for dapsone in bovine muscle. Bond Elut Plexa SPE, combined with LC/MS/MS, enables sensitive quantitation of dapsone in meat samples at sub-ppb concentrations.

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