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

Application Note

Food Testing and Agriculture

Authors

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Introduction

In this study, bovine muscle was prepared and analyzed for dapsone at the sub-ppb level, using a QuEChERS methodology with Agilent Bond Elut QuEChERS kits for veterinary drugs, efficient separation with an Agilent Poroshell 120 LC column, and sensitive detection with an Agilent 6460 Triple Quadrupole LC/MS system.

Material and Methods

Sample preparations

Agilent Bond Elut QuEChERS extraction salt packet for veterinary drugs (p/n G7750-10001)

Agilent Bond Elut QuEChERS dispersive SPE for veterinary drugs, 15-mL tube (p/n 5982-4980)

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:	% B, linear to 80 % B in 8 min
Temperature:	Ambient
Sample vials:	Agilent Certified Vials (p/n 5183-2072)
System:	Agilent 1260 Infinity LC

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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

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

Sample comminution

Bovine muscle was cut into small pieces and placed into a clean plastic bag and frozen at –20 °C overnight. The following day, only the required amount of frozen muscle was removed and thoroughly blended. Samples were comminuted thoroughly for sample homogeneity. It was verified that no pieces of muscle were visible in the final sample.

Extraction/partitioning

A 5 g (± 0.1 g) amount of homogenized sample was placed into a 50-mL centrifuge tube. QC samples were fortified with 20 µL of appropriate QC spiking solution. IS spiking solution (100 µL) was added to all samples except the control blank. Tubes were capped and vortexed for 1 minute. A 10-mL aliquot of water was added to each tube using a dispenser. Tubes were capped and vortexed for 1 minute. A 15-mL aliquot of ACN was then added to each tube using a dispenser. An Agilent Bond Elut QuEChERS extraction salt packet for veterinary drugs was added directly to each tube. Tubes were sealed tightly and shaken vigorously for 20 seconds by hand to ensure that the solvent interacted well with the entire sample and crystalline agglomerates were broken up sufficiently. Sample tubes were centrifuged at 4,000 rpm for 5 minutes.

Dispersive SPE cleanup

An 8-mL aliquot of the upper ACN layer was transferred into Agilent Bond Elut QuEChERS dispersive SPE for veterinary drugs 15-mL tubes. The tubes were capped tightly and vortexed for 1 minute. The tubes were centrifuged with a standard centrifuge at 4,000 rpm for 5 minutes. A 5-mL portion of the extract was transferred into a tube and dried under nitrogen below 40 °C. The resulting residue was dissolved and made to a constant volume of 1 mL using ACN:water (1:9). Then, the residue was filtered through a 0.2-µm filter membrane (p/n 5190-5106) and analyzed with LC/MS/MS.

Results and Discussion

Linearity and limit of detection

Solutions used to create external calibration curves were prepared by using a combined working solution to spike 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 (S/N) 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 = 477.34x-28.94	0.999	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 QuEChERS (post-spiked samples). Figure 1 shows a chromatogram obtained from the analysis of bovine blank sample spiked with low level of dapsone. Figure 2 is the sample blank.

Table 3. Extraction recoveries of dapsone from bovine muscle with QuEChERS.

Compound	Recovery (RSD) n = 6, 0.5 µg/kg	Recovery (RSD) n = 6, 1 µg/kg	Recovery (RSD) n = 6, 2 µg/kg
Dapsone	78.3 (7.6)	77.5 (4.7)	82.2 (5.9)

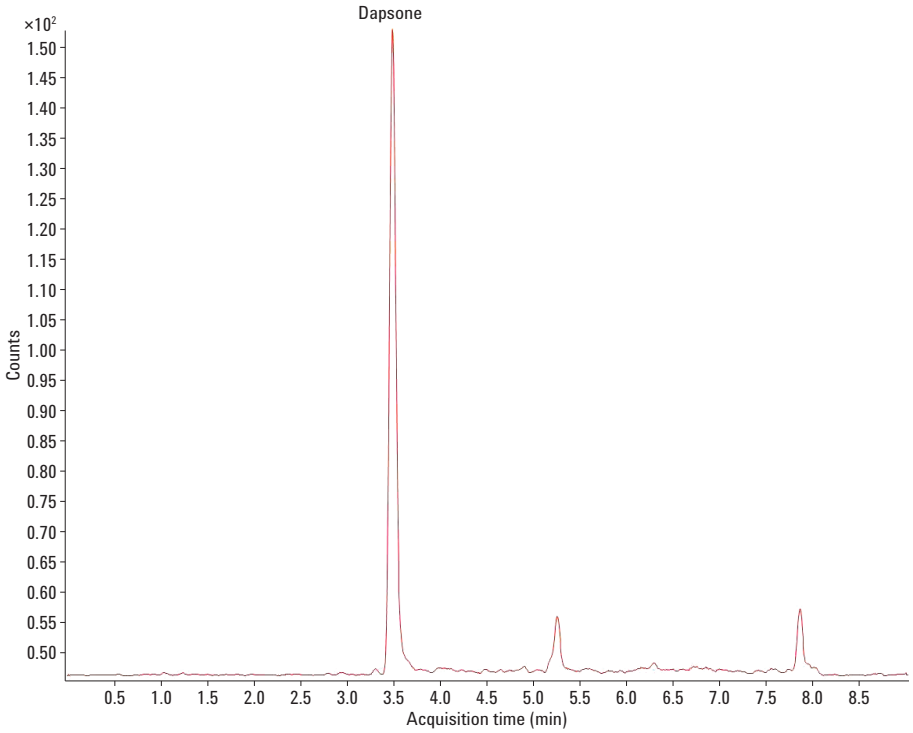

Figure 1. Chromatogram of dapsone obtained from bovine muscle spiked with a level at 1 µg/kg.

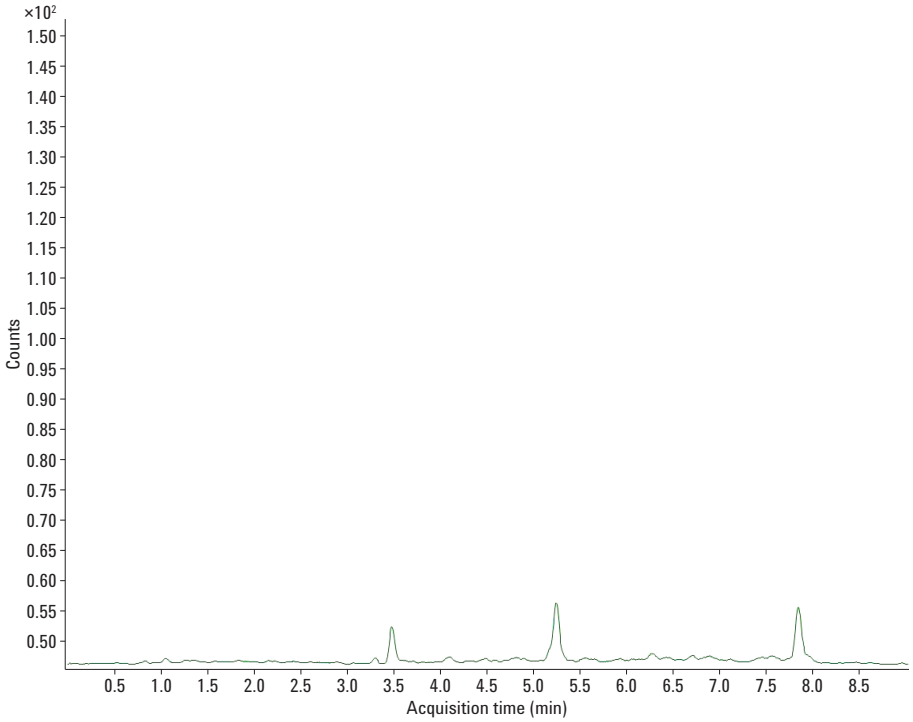

Figure 2. Chromatogram of dapsone obtained from a bovine muscle blank sample.

Conclusions

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

For More Information

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