DIONEX

Application Note 353

Rapid Determination of Sulfonamide Residues in Animal Tissue and Infant Food Containing Animal Products Using Accelerated Solvent Extraction (ASE)

INTRODUCTION

Veterinary drugs containing antimicrobial agents are often administered to livestock for the treatment or prevention of disease and, at low levels, to promote growth in food-producing animals. Recently, government agencies have discovered that some livestock companies are abusing the use of these antimicrobial drugs, by administering them at higher than recommended levels to promote faster growth. This practice can result in unwanted residues of these drugs in meat and meat products eaten by humans. Negative health effects in humans have been traced to the consumption of these antimicrobial drugs and their metabolites. Therefore, screening for these types of residues in animal tissue and meats has become a priority, not only in the United States, but in Europe as well.

Sulfonamides are one class of antimicrobial agent used widely in the livestock industry to promote growth. Sulfonamides are often overused because they are inexpensive and readily available. Short-life sulfonamides are mixed with the feed several times per day to prevent bacterial contamination, while the long-life sulfonamides are injected into the animals at high levels to increase animal growth. American and European institutions have established maximum residue levels (MRLs) to regulate the amount of veterinary medicinal product residues allowed in meat and meat products used for human consumption.

This application note shows that ASE® is an excellent technique for the extraction of sulfonamides from meat and baby food containing meat products.

EQUIPMENT

ASE 200 Accelerated Extractor* with Solvent Controller (P/N 048765)11-mL Stainless Steel Extraction Cells (P/N 055422)

Glass-Fiber Filters (P/N 049458)

Collection Vials, 40 mL (P/N 048783)

Analytical Balance (to read to nearest 0.0001 g or better)

Standard-Grade tissue homogenizer

Centrifuge (any standard laboratory centrifuge capable of at least 10,000 rpm)

Freezer capable of –18 °C

**ASE 150 and 350 can be used for equivalent results*

REAGENTS

C18 resin (can be purchased from any reputable manufacturer like Supelco or Restek)

STANDARD REFERENCE MATERIAL

Sulfamethoxazole (SMX), Sulfamoxole (SMO), Sulfapyradine (SPD), Sulfamethoxypiridazine (SMT), Sulfachloropyridazine (SCP), Sulfamethoxypiridazine (SMP), Sulfadiazine (SDZ), Sulfamerazine (SMR), Sulfamethazine (SMZ), Sulfasomidine (SIM), Sulfamonomethoxine (SMM), Sulfadimethoxine (SDM), Sulfaquinozaline (SQX)

SAMPLES

Crude Meat Samples

Bovine: Tissues of veal, tender beef, and beef

Porcine and Poultry: Ham and chicken

Baby Food Samples

Containing: Bovine (veal and beef), porcine (pork products and ham), poultry (chicken and turkey)

SOLVENTS

HPLC-grade water

EXTRACTION CONDITIONS

Solvent: HPLC-grade water

Temperature: 160 °C

Pressure: 1500 psi*

Static time: 5 min

Static cycles: 1

Flush: 60%

Purge: 60 s

**Pressure studies show that 1500 psi is the optimum extraction pressure for all ASE applications.*

SAMPLE PREPARATION

Baby Food Samples

Mix 2 g of baby food with 4 g of C18 material using a mortar and pestle until the entire mixture is of uniform consistency. Transfer this mixture to an 11-mL cell containing a glass-fiber filter.

Crude Meat Samples

Homogenize the meat samples using any standard tissue homogenizer. This task should be done with deionized (DI) water added to the sample, starting the homogenizer at 5000 rpm and increasing to 25,000 rpm for 15 min. Evaporate excess water. Weigh out approximately 2 g of homogenized tissue and mix with 4 g of C18 resin until the entire mixture is of uniform consistency. Transfer this mixture to an 11-mL cell containing a glass-fiber filter.

Extraction Procedure

Place the cell onto the ASE 200 cell tray. Label the appropriate number of collection vials and place these into the vial carousel. Set up and load the method suggested above. Then press start. After the extraction, allow the sample vials to cool in a freezer at –18 °C for one hour to precipitate the coextracted lipids. Centrifuge the vials for 5 min at 10,000 rpm. Inject 100 µL of the supernatant for analysis.

Analytical Procedure

For the data shown in Table 1, all analyses were performed using LC-MS/MS. The HPLC column was an Alltima™ 25 cm × 4.5 i.d. column filled with 5 µm C-18 reverse-phase packing. For additional details, consult Reference 1.

RESULTS AND DISCUSSION

When compared with diatomaceous earth, C18 material was the best agent for dispersing the meat samples because it retained more of the lipids from the sample matrix, giving cleaner extracts.[1]

Temperature tests were conducted and the best recoveries of all sulfonamides were at 160 °C with no negative effects on analyte stability.[1]

Recovery levels were tested on bovine, porcine, and poultry samples. Table 1 lists the recovery results with the limits of detection (LODs) and limits of quantitation (LOQs) listed in Table 2.

CONCLUSION

For several reasons, ASE technology has proven advantages for extracting sulfonamides from meat. First, extraction times of ~15 min when using ASE, second, ASE uses between 25–30 mL of solvent, and third, because of the efficiency offered by the increased temperature and pressure, ASE is able to extract polar compounds at acceptable recovery levels such as sulfonamides using water as the extraction solvent, which cuts solvent purchase and disposal costs.

ACKNOWLEDGEMENTS

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LIST OF SUPPLIERS

Supelco Inc, Supelco Park Bellefonte, PA 16823

Table 1. Recoveries (%) of Sulfonamides from Various Meat Matrices Spiked with 100 ppb Standards						
	Bovine Meat		Porcine Meat		Poultry Meat	
Analyte	Raw Meat	Baby Food	Raw Meat	Baby Food	Raw Meat	Baby Food
SPD	89 (5)	90 (4)	92 (5)	94 (3)	91 (4)	93 (3)
SDZ	92 (4)	91 (3)	93 (4)	95 (4)	94 (3)	94 (4)
SMX	92 (4)	94 (3)	94 (5)	93 (3)	96 (4)	95 (3)
SMR	99 (4)	101 (4)	98 (5)	99 (4)	98 (6)	100 (5)
SMO	70 (6)	71 (4)	72 (5)	74 (5)	76 (5)	75 (5)
SMT	86 (6)	87 (6)	90 (5)	92 (5)	91 (4)	93 (4)
SIM	94 (4)	97 (4)	98 (5)	100 (4)	99 (3)	98 (4)
SMZ	88 (5)	91 (5)	90 (5)	89 (4)	92 (4)	95 (4)
SMP	85 (6)	84 (4)	85 (5)	85 (5)	85 (5)	87 (3)
SMM	90 (6)	92 (5)	92 (5)	91 (5)	94 (5)	95 (5)
SCP	79 (4)	83 (5)	85 (5)	84 (6)	82 (6)	88 (4)
SQX	81 (5)	84 (4)	82 (6)	82 (5)	85 (5)	87 (5)
SDM	85 (6)	88 (5)	90 (5)	92 (5)	93 (4)	91 (4)

The numbers in parenthesis equal total extractions performed.

Table 2. LODs and LOQs of the Method for Analyzing Sulfonamides in Beef, Raw Meat, and Baby Food				
	Raw Meat		Baby Food	
Analyte	LOD (ppb)	LOQ (ppb)	LOD (ppb)	LOQ (ppb)
SPD	1.4	4.2	1.2	3.5
SDZ	1.6	4.8	0.8	2.4
SMX	2.6	7.8	1.4	4.2
SMR	1.9	5.7	1.5	4.5
SMO	1.1	3.3	1.1	3.3
SMT	1.1	3.3	0.4	1.2
SIM	1.7	5.1	1.7	5.1
SMZ	2.1	8.3	1.6	4.8
SMP	0.7	2.1	0.4	1.2
SMM	0.9	2.7	0.9	2.7
SCP	1.3	3.7	0.5	1.5
SQX	1.2	3.8	1.2	3.8
SDM	0.8	1.8	0.5	1.5
LOD = Three × the noise level of the baseline in the chromatogram (S/N = 3)				

LOQ = Three × the LOD. The noise level varies depending on the sample matrix, therefore there are different LODs for different samples.[1]

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Sigma-Aldrich Chemical Company, 3050 Spruce St., St. Louis, MO 63103 USA, Tel: 800-325-3010, www.sigmaaldrich.com.

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