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Determination of sulfadimethoxine and ^4N-acetylsulfadimethoxine in bovine plasma, urine, oral fluid, and kidney and liver biopsy samples obtained surgically from standing animals by LC/MS/MS

Hui Li*, Michelle L. Smith, O. Alberto Chiesa[1], Philip J. Kijak

Food and Drug Administration, Center for Veterinary Medicine, 8401 Muirkirk Road, Laurel, MD 20708, USA

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ABSTRACT

A quantitative method was developed and validated to measure the concentration of sulfadimethoxine (SDM) and its major metabolite, ^4N-acetylsulfadimethoxine (AcSDM), in bovine tissues and body fluids. Liquid chromatography/tandem mass spectrometry (LC/MS/MS) gave quantitative results for these two analytes in extracts from bovine plasma, urine, oral fluid, kidney, and liver, using SDM-d_4 as internal standard (I.S.). The lower limit of quantitation (LLOQ) for both analytes in these matrices was validated at 2, 100, and 5 ng/mL in plasma, urine, and oral fluid respectively, and 10 ng/g in both kidney (cortex) and liver. The overall accuracy (average of 4 levels) is, for plasma, 104% (SDM) and 95% (AcSDM), with standard deviation of 9% (SDM) and 15% (AcSDM); for urine, 100% (SDM) and 106% (AcSDM), with standard deviation of 5% (SDM) and 6% (AcSDM); for oral fluid, 103% (SDM) and 103% (AcSDM), with standard deviation of 4% (SDM) and 4% (AcSDM); for kidney, 101% (SDM) and 111% (AcSDM), with standard deviation of 7% (SDM) and 6% (AcSDM); and for liver, 99% (SDM) and 115% (AcSDM), with standard deviation of 11% (SDM) and 9% (AcSDM). C18 SPE cartridges were used to clean-up these matrices, except for urine which was diluted directly with buffer before analysis by LC/MS/MS.

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1. Introduction

The use of sulfadimethoxine (SDM) in meat and milk-producing cattle has been approved by U.S. Food and Drug Administration (FDA) for treatment of shipping fever complex, bacterial pneumonia, calf diphtheria, and foot rot. This synthetic drug is effective against bacterial and coccidial infections, and can be administered orally [1] or intravenously [2]. Currently in U.S. the residue tolerance of SDM in edible tissue of cattle is 0.1 parts per million (ppm; 0.01 ppm in milk) [3].

Monitoring the proper use of animal drugs to ensure food safety is one important role of U.S. FDA. If SDM is not administered correctly to cattle or used off-label, elevated drug residue concentration may occur in edible tissues. The emergence of drug-resistant microbes to SDM is another public health concern [4–7]. Currently, the U.S. Department of Agriculture (USDA) uses the Fast Antibiotic Screen Test (FAST), a microbiology-based assay, for in-plant screening to determine the presence of antibiotics including SDM [8,9]. Bovine kidney is the primary monitoring site for the presence of antibiotics, which is only available after the animal is dead. The slaughter of animals containing residues can result in unsafe food entering the Nations food supply or the loss of a carcass. This can easily be avoided by extending the period the treated animal is held before slaughter. Even though the drug depletion time has been established, SDM residues are still a significant cause for carcass condemnation due to off label use. A simple testing method using easily obtainable animal fluid (serum, urine, saliva, etc.) to evaluate drug residue level is desirable. An earlier study has demonstrated that a tissue–fluid correlation exists for sulfamethazine residues in swine [10]. However, information is not available to develop a correlation of SDM residues in bovine tissues. The FDA Center for Veterinary Medicine (CVM) is conducting research to develop tissue–fluid correlations for a variety of veterinary drugs, such as penicillin G [11], gentamicin [12], and SDM in cattle.

Analytical methods were needed to determine the residual level of SDM and its major metabolite in bovine species, ^4N-acetylsulfadimethoxine (AcSDM) [13], to support the tissue–fluid correlation research. The target tissues and fluids from Holstein

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* Corresponding author.
E-mail address: hui.li@fda.hhs.gov (H. Li).
[1] Present address: FDA Center for Devices and Radiological Health, Laurel, MD, USA.

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steers included kidney, liver, plasma, urine, and oral fluid. SDM is a chemically stable compound with a depletion half-life around 8–12.5 h in bovine species, if given intravenously [14,15]. Procedures are available from both published literature and government agencies on extracting and analyzing SDM in animal tissues and fluids from a variety of bovine matrices. Organic solvents such as ethyl acetate, methanol, and acetonitrile have been used for extraction, followed by either a solid phase extraction (SPE) or a liquid–liquid extraction (LLE) step for clean-up, and combined with the use of LC-UV, LC-fluorescence, or LC/MS/MS for detection and quantification [16–19]. Alternative methodologies like supercritical fluid extraction (SFE) and online SPE were also reported [20,21]. The collection of published methods provided valuable references for developing a “fit-to-purpose” method to achieve the goal of this study, which was to quantify both SDM and AcSDM over a wide concentration range in a variety of tissues and fluids. As part of experimental design, some of the tissue samples were acquired by the use of a laparoscopic procedure [22], to greatly reduce the number of animals in a depletion study. The design allowed multiple organ biopsy samples from an individual steer at designated time intervals to be taken before the animal was euthanized. However, the biopsy samples obtained as such would be of limited quantity and rather variable in weight (ca. 50–200 mg), which called for a method with high sensitivity and low susceptibility to matrix effect. The sample extraction and analysis throughput needed to be reasonably high to assay the large number of samples from multiple tissues and fluids.

Based on the experience at CVM in quantifying penicillin G and gentamicin in small size (ca. 100 mg) bovine kidney and liver biopsy samples [23,24], an LC/MS/MS method for quantifying SDM and AcSDM, using SPE for tissue and plasma clean-up and direct buffer dilution for urine sample preparation, has been developed and validated. The method contains sample extraction/clean-up procedures customized to each matrix, while it employs the same analytical equipment and instrumental conditions for all extracts. This method has been demonstrated to be sensitive, precise, and practical, to provide data for the continuing tissue–fluid correlation study.

2. Experimental

2.1. Apparatus

The LC/MS/MS system consists of a Waters Quattro Micro API bench-top triple quadrupole mass spectrometer (Milford, MA), and an Agilent 1100 LC system, including a binary pump, an autosampler with refrigerated sample tray, and a column compartment with temperature control (Santa Clara, CA). A Thermo BDS Hypersil C18 LC column, 150 mm × 2.1 mm ID, particle size 5 μm silica, fitted with Thermo C18 guard column, was used throughout the study (Thermo Fisher, Waltham, MA). A Sorvall 3C centrifuge (floor model) was used for centrifugation (Dupont Company, Wilmington, DE). The pH of buffer solutions was measured with a Fisher Accumet Research AR15 pH meter (Fisher Scientific, Pittsburgh, PA) equipped with an Accumet Ag/AgCl single junction probe. Liquid samples or extracts were accurately measured and transferred with calibrated variable micropipettes of proper capacity (Eppendorf North America, Westbury, NY). Organic solvents were measured with disposable graduated glass pipettes (Fisher Scientific). An Omni TH tissue homogenizer (Omni International, Marietta, GA) fitted with disposable plastic probes (7 mm × 110 mm) was used for blending kidney or liver lumps of approximately 100 mg. Biopsy samples were collected in pre-weighed round-bottom Falcon 14-mL centrifuge tubes (Becton Dickinson and Company, Franklin Lakes, NJ). PVDF filters with 0.22 μm pore size and 13 mm diameter (MilliPore, Billerica, MA) were used to filter the extract into 1.5-mL, amber glass autosampler vials. Varian Bond Elut C18 SPE cartridges (500 mg absorbent, 3 mL capacity, Varian Inc., Palo Alto, CA) were used to clean-up the raw sample extract.

2.2. Reagents and standards

Sulfadimethoxine was obtained from Sigma–Aldrich (St. Louis, MO) with 99% purity. Sulfadimethoxine-d_4 was purchased from Toronto Research Chemicals (Toronto, Canada) with overall purity of 98% (isotopic purity 99%). AcSDM was synthesized in-house from SDM according to a published procedure [25]. This compound was characterized with ^{1}H NMR and high resolution MS for identity, and its purity was evaluated with NMR and LC-UV (265 nm). No detectable impurities were found. Ammonium formate, ammonium acetate, and trichloroacetic acid (TCA) were purchased from Sigma–Aldrich with analytical or higher purity. Other reagents were ACS grade formic acid (88%) from Mallinckrodt (Phillipsburg, NJ), glacial acetic acid and ammonia (~30%) from Fisher Scientific, and HPLC Grade acetonitrile and methanol from Burdick and Jackson (Morristown, NJ). Deionized (D.I.) water was produced in-house with the Milli-Q system (MilliPore, Billerica, MA) with a resistivity ≥18 MΩ cm.

2.3. Solutions

Stock solutions for SDM, AcSDM, and SDM-d_4 were made at approximately 1 mg/mL (weighed about 10 mg and recorded exactly to 0.01 mg) in 10 mL methanol in a volumetric flask. A 200 μg/mL working stock solution for each of SDM and AcSDM, and a 5 μg/mL solution for SDM-d_4, was then prepared by diluting a calculated amount of the 1 mg/mL stock solution with methanol. A series of mixed stock solutions containing an equal amount of SDM and AcSDM were prepared by mixing and diluting proper volumes of the 200 μg/mL stock solutions, or from a mixed stock solution of a greater concentration. These standard solutions were store at <−10 °C, and the analytes were found to be stable over a period of 6 months. The long time exposure to light was avoided while these stock solutions were in use.

A 0.05 g/mL TCA solution was prepared by dissolving 50.0 g TCA in D.I. water to make a 1 L solution. A 50 mM ammonium formate buffer solution (AFB) was prepared by dissolving 6.50 g ammonium formate (97%) in D.I. water to make a 2 L solution. The pH was then adjusted to 3.9 ± 0.1 with formic acid. A 50 mM ammonium acetate buffer (AAcB) was prepared by dissolving 15.9 g ammonium acetate (97%) in D.I. water to make a 4 L solution, and the pH was adjusted to 6.0 ± 0.1 with glacial acetic acid. LC mobile phase A consisted of 5% acetonitrile with 0.1% formic acid in D.I. water (v/v), prepared by mixing 200 mL acetonitrile, 3.8 L Milli-Q water, and 4.55 mL 88% formic acid. This solution was filtered through 0.2 μm Nylon disk before use.

2.4. Biological samples storage, and stability of analytes in matrices and extracts

The test system consisted of the following bovine tissues and body fluids: kidney (cortex), liver, plasma, urine, and oral fluid. Samples of these tissues and fluids were collected from 9 Holstein steers. The details of dosing and sample collection will be published separately [22]. Blank control kidney and liver were obtained either from stored samples generated previously known to be free of SDM, or from groceries and a farmer's market, which were screened to be free of detectable drug residues before use. Blank control urine, plasma, and oral fluid were obtained from each individual animal before dosing. All raw samples and extracts were stored at <−70 °C. The majority of the incurred samples were extracted and analyzed within a few days to a few weeks after collection.