A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE
DETERMINATION OF NINE SULFONAMIDES IN MILK

Vipin K. Agarwal
The Connecticut Agricultural Experiment Station, Box 1106, New Haven, CT 06504

ABSTRACT

A High Performance Liquid Chromatographic (HPLC) method for the determination of sulfadiazine, sulfathiazole, sulfapyridine, sulfamerazine, sulfamethiazole, sulfamethazine, sulfachloropyridazine, sulfadimethoxine, and sulfaquinoxaline in milk is described. Milk (10mL) is extracted three times (50, 40, and 40mL) with chloroform/acetone, the extract evaporated to dryness, and redissolved in 5mL potassium phosphate buffer (1.0 molar, pH 4.4). Hexane (5mL) is added to extract the lipid materials. The aqueous buffer layer containing sulfonamides is passed through a cyclobond-I solid phase extraction (SPE) cartridge and the cartridge is washed with 5mL potassium phosphate buffer. The sulfonamides, which are retained on the cartridge, are then eluted with 4mL aqueous acetonitrile (20% water). The eluent is evaporated to remove acetonitrile and the final volume of the aqueous eluent is made to 4mL with ammonium acetate buffer. The eluent is analyzed by HPLC using a reverse phase column with UV detection at 265nm. The recoveries of individual sulfonamides ranged from 64.0% to 85.9% in samples fortified at 10 and 20ppb levels.

INTRODUCTION

The use of sulfonamides as veterinary drugs for the treatment of a variety of bacterial infections is very common. In food producing animals, sulfonamides are used not only for therapeutic purposes but also for prophylactic purposes.

There has been more concern lately about the residues of sulfamethazine in milk since a study by the Food and Drug Administration's National Center for Toxicological Research (NCTR) indicated that it may be a carcinogen (1). Residues of sulfonamides in foods can be a health hazard to consumers (2). Firstly, the carcinogenicity of some sulfonamides such as sulfamethazine may be a serious concern (2). Secondly, continuous exposure of certain microorganisms to these drugs may result in the development of drug resistant strains (2). In the past, residues of sulfa drugs have been found in milk offered for sale to the consumers. A nationwide survey by the Food and Drug Administration in 1988 reported that 45% of the milk...
samples contained detectable amounts of sulfamethazine (3). In another survey, which included 30 samples from 10 cities across Canada, two contained sulfamethazine residues at 11.40 and 5.24 ppb levels (4).

The U. S. Food and Drug Administration has, therefore, set tolerance limits of sulfonamides in milk. Presently, sulfadimethoxine is the only sulfa drug allowed for use in lactating animals and the residues of total sulfonamides, including sulfadimethoxine, should not exceed 10 ppb in milk (5).

Sulfonamide residues in milk have been determined by various techniques which include immunoassay (6), microbiological (7), thin layer chromatography (TLC) (8-10), gas chromatography (11), and high performance liquid chromatography (12-20).

High performance liquid chromatography has become the most widely used technique for the analysis of sulfa drug residue in milk and a number of methods have been described (12-20). In general all HPLC methods which can detect sulfonamides to 10 ppb level require extensive cleanup steps before HPLC analysis.

An HPLC method developed by Smedley and Weber (16) has successfully been applied for the detection of ten sulfonamides in milk to 10 ppb level and is presently being used by the FDA for testing milk. This method involves a chloroform/acetone extraction followed by partitioning of the extract between hexane and potassium phosphate buffer before HPLC analysis. A number of extraneous peaks were, however, present in the chromatogram which could make quantitation difficult. Also, two separate chromatographic conditions are used for the determination of ten sulfonamides.

In this report an HPLC method for the quantitative determination of nine sulfonamides at a low level of 10 ppb is described.

EXPERIMENTAL

Reagents
(a) Sulfonamides: Sulfadiazine (SDZ), Sulfathiazole (STZ), Sulfapyridine (SPD), Sulfamerazine (SMR), Sulfamethazole (SMTZ), Sulfamethazine (SMZ), Sulfachloropyridazine (SCP), Sulfadimethoxine (SDM), Sulfaquinoxaline (SQX) (Sigma Chemical Co., St. Louis, MO).

(b) Potassium phosphate buffers (mono and dibasic), ammonium acetate, acetic acid, chloroform, acetone and HPLC grade methanol (Fisher Chemical Co., Fairlawn, NJ).

(c) Potassium phosphate buffer: Dissolve 13.60 gm monobasic potassium phosphate buffer in 100 mL distilled water.

(d) Ammonium acetate buffers: (1) To prepare a 25 millimolar ammonium acetate buffer, dissolve 1.95 gm ammonium acetate in 900 mL distilled water, adjust pH to 4.7 with acetic acid and make final volume to 1000 mL. (2) Dissolve 1.95 gm ammonium acetate in 900 mL distilled water, adjust pH to 8.0 with ammonium hydroxide and make final volume to 1000 mL.

(e) Mobile phase for HPLC:
Solvent A: Ammonium acetate buffer a(pH 4.7):methanol (850:150).