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Note

Determination of sulfanilic acid in the presence of sulfanilamide and some sulfa drugs by reversed-phase ion-pair high-performance liquid chromatography

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Interest in finding methods for the detection and quantitation of sulfa drug residues in animal feeds and tissue is considerable. One approach under investigation in our laboratory involved hydrolysis of the drugs to a common product. This would permit the development of a rapid screening procedure applicable to all members of the class of sulfa drugs. In order to study the efficiency of various hydrolysis methods by high-performance liquid chromatography (HPLC), one must separate sulfanilic acid (SAA) from another hydrolysis product, sulfanilamide (SAM), and from different sulfa drugs.

Other researchers have described the separation of sulfonamides and their metabolites in urine¹ and in the presence of other drugs² on amino-bonded reversed-phase HPLC columns. Karger and co-workers^{3,4} demonstrated the versatility and efficiency of ion-pair chromatography for sulfa drugs of widely varying pK_a values and hydrophobicity. However, no information is available for separating SAA from SAM and sulfa drugs by HPLC. In this paper, we describe such a method for the detection and quantitation of some sulfa drugs following hydrolysis to a compound common to all the drugs.

EXPERIMENTAL*

Reagents

Sulfamerazine [SMER; 4-amino-N-(4-methyl-2-pyrimidinyl)benzenesulfonamide], SAM, SAA and sulfathiazole (STHA; 4-amino-N-2-thiazolylbenzenesulfonamide) were purchased from Sigma (St. Louis, MO, U.S.A.), sulfamethazine [SMET; 4-amino-N-(4,6-dimethyl-2-pyrimidinyl)benzenesulfonamide] from American Cyanamid (Princeton, NJ, U.S.A.); sulfadimethoxine [SDMX; 4-amino-N-(2,6-dimethoxy-4-pyrimidinyl)benzenesulfonamide] from Hoffmann-La Roche (Nutley, NJ, U.S.A.); glacial acetic acid from J. T. Baker (Phillipsburg, NJ, U.S.A.); tetrabutylammonium hydroxide from Aldrich (Milwaukee, WI, U.S.A.); and methanol (distilled from glass) from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). All reagents were used as received without further purification.

* Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Apparatus

The HPLC system consisted of two LDC (Riviera Beach, FL, U.S.A.) Constametric pumps controlled by a Gradient Master programmer and connected to a UV III Monitor, which was operated at 254 nm for detection. Sample introduction was achieved with a Rheodyne 7120 sampling valve.

Operating conditions

A Waters Assoc. (Milford, MA, U.S.A.) μ Bondapak C_{18} column (30 cm \times 4 mm I.D.) was eluted isocratically at a flow-rate of 1 ml/min with a mobile phase of water-methanol-acetic acid (85:15:0.5) to which tetrabutylammonium hydroxide (0.1 %) was added as the ion-pairing reagent.

Method: sulfamethazine hydrolysis

A 260- μ g portion of SMET was added to 2 ml of 2.5 M sulphuric acid and heated for 1 h at 100°C. After cooling, the solution was made alkaline (pH 8) with ammonium hydroxide, and the excess of ammonia was removed under a stream of nitrogen at 40°C; the volume was adjusted to 5 ml before HPLC analysis.

RESULTS AND DISCUSSION

Use of the separation of hydrolysates of sulfa drugs by HPLC as an analytical technique requires that the hydrolysis products SAA and SAM be retained on the column and completely resolved from each other as well as from the original drug. In the conventional adsorption mode on a 10- μ m silica column, a mobile phase that dissolved all the components and gave an adequate separation could not be obtained. SAA was not adequately retained on a strong cation-exchange column with methanol-water as solvent system and varying the pH and ionic strength.

On reversed-phase HPLC of a mixture of sulfa drugs (SMER and SMET), SAA, and SAM, the SAA was poorly retained (t_0 ca. 2.5 min) and was not completely resolved from SAM (Fig. 1, trace A). Addition of the ion-pairing reagent tetrabutylammonium hydroxide to the mobile phase increased the retention of SAA with little effect on the retention of the other components in the mixture (Fig. 1, trace B). STHA (t_R 10.5 min) and SDMX (t_R 47.3 min) behave similarly. This is attributed to the formation of the SAA-counter-ion complex at pH 3.5 and to the fact that there is no ion pairing with the other components. The pH of the system is considerably below that required for the formation of ion-pairs of SAM (pK_a 10.4), SMET (pK_a 7.6), SMER (pK_a 6.9), STHA (pK_a 7.2) and SDMX (pK_a 6.0). This system was suitable for separating these sulfa drugs and their hydrolysis products. SMET was chromatographed both before (Fig. 2, trace A) and after partial acid hydrolysis (Fig. 2, trace B). SMET, SAM, and SAA are completely resolved and can be readily quantitated.

The presence of salt, resulting from neutralization of the hydrolysis medium, affects the retention and peak size of the components in the mixture. Therefore, quantitation was achieved by comparing peak heights of the sample with those of standard solutions of equal salt concentration. The calibration curves were linear over a range of 0 to 0.7 μ g.

Preliminary investigations indicate that gradient elution is possible for more difficult separations of other sulfa drugs using this system. This method is currently being applied to the hydrolysis of sulfa drugs in edible meat tissue.

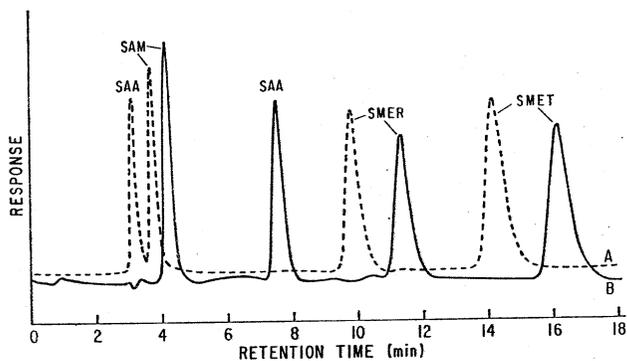


Fig. 1. Reversed-phase chromatogram of 0.5 μg each of SAA, SAM, SMER and SMET. Trace A, without ion-pairing; trace B, with ion-pairing.

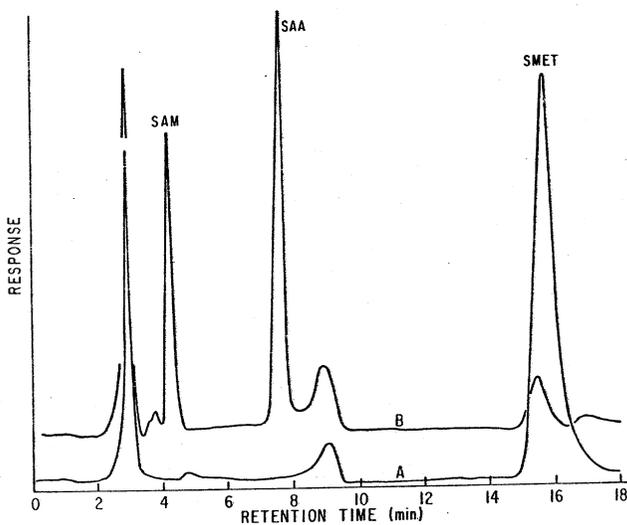


Fig. 2. Reversed-phase ion-paired chromatogram of 0.26 μg of SMET at pH 3.5. Trace A, before acid hydrolysis; trace B, after acid hydrolysis.

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