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Short communication

Simultaneous determination of seven sulfonamide residues in swine wastewater by high-performance liquid chromatography

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Abstract

A HPLC–UV method was used for simultaneous determination of the residual sulfonamides in swine wastewater. Sulfonamides were extracted with ethyl acetate prior to chromatographic analysis. An ODS column was used for separation and UV detection was used to monitor the sulfonamides. Factors that affect the chromatographic behavior and the recovery have been studied. Calibration graphs were linear with very good correlation coefficients ($r>0.9999$) from 0.05 to 10 μg/ml. The applicability of the method to the analysis of swine wastewater samples was demonstrated. © 1998 Elsevier Science B.V.

Keywords: Water analysis; Sulfonamides

1. Introduction

Sulfonamides are used extensively in food-producing animals for therapeutic and prophylactic purposes [1,2]. However, only juvenile-swine are permitted to be treated with sulfonamides to prevent sulfamide residues in swine tissue food products. In addition to the notable residues of antibiotics in animal tissues, large amounts of antibiotics and their metabolites may also be egested in the faeces which could inhibit the growth of microorganisms involved in a biological process for wastewater treatment. Therefore, knowing the concentrations of residual sulfonamides in swine wastewater and then selecting an appropriate strategy are required to obtain a high treatment efficiency.

Several methods have been developed to detect sulfonamides in animal tissues [3–12]. Although microbiological methods and colorimetric methods were convenient, they were inappropriate in terms of sensitivity and selectivity [2–4]. Chromatographic methods were thus developed [5–12]. Among them, thin-layer chromatography (TLC) was subjected to the disadvantage of low recovery and poor reproducibility [5,6] and gas chromatography–mass spectrometry (GC–MS) suffered from the low volatility of sulfonamides. Moreover, the expensive MS detector is not present in many routine analytical laboratories [7]. Recently, high-performance liquid chromatography (HPLC) methods have been used to successfully determine sulfa drugs in feeds and animal tissues [8–12]. However, there was not an effective method available for measuring the sulfonamides in swine wastewater.

This paper describes the HPLC procedure which enables one to simultaneously determine seven sul-

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fonamides and the internal standard in highly complex swine wastewater. The optimum chromatographic conditions such as selection of detection wavelength, the pH and the concentration of buffer solution, as well as some interferences were studied.

2. Experimental

2.1. Apparatus

The HPLC system used in this work was a Gilson system (Villiers-le-Bel, France), and a Waters 484 UV tunable detector (Milford, MA, USA) with a 20 μl flow cell. The detection wavelength was set at 260 nm. A reversed-phase Inertsil ODS-2 column (15 cm×4.6 mm I.D., 5 μm particle size) (Vercopak) was used for all separations. A Rheodyne 7125 injector (Cotati, CA, USA) with a 20 μl external loop was used for sample introduction. A Chromatocorder 12 Integrator (SIC, Japan) was used to obtain the chromatogram and perform data calculations.

2.2. Chemicals and reagents

Deionized water was produced using a Barnstead Nanopure water system (Thermolyne, Dubuque, IA, USA) for all aqueous solutions. All chemicals and solvents were of ACS reagent grade. Stock solutions of 1000 mg/l sulfonamides, sulfadiazine (SDIA), sulfathiazole (ATHA), sulfamerazine (SMEA), sulfamethazine (SMAZ), sulfacetamide (SAAM), sulfamethoxazole (SMOX), sulfamonomethoxine (SMON) and nicotinamide, were prepared by dissolving 0.100 g in 5 ml 0.01 *M* HCl, and diluted with water to 100 ml. The solutions were stored in brown glass bottles, and kept at 5°C for a maximum of three months. Fresh working solutions were prepared daily by appropriate dilution of the stock solutions. The hydrochloric acid and the glacial acetic acid was obtained from Baker (Phillipsburg, NJ, USA).

Ethyl acetate and methanol were HPLC grade obtained from Mallinckrodt (KY, USA). The HPLC eluent was prepared as 20% (v/v) of methanol and 0.1 *M* of sodium acetate (Riedel-de Haën, Hannover, Germany). Acetic acid (0.01 *M*) (Riedel-de Haën) was used to adjust the pH. All eluents were filtered through a 0.45 μm poly(vinylidene difluoride) (PVDF) membrane filter and degassed ultrasonically.

2.3. Procedure

Extraction of sulfonamides was achieved by introducing a 50 ml portion of wastewater sample (adjusted pH to 6.6 with acetic acid) into a 250 ml separating funnel, followed by a 5 ml of nicotinamide solution and 50 ml of ethyl acetate. The mixture was shaken vigorously for 5 min and the vapor exhausted in the funnel once every five times shaking. After standing the mixture for 1 min, the ethyl acetate layer was transferred into a 150 ml flask. The residual aqueous layer was extracted further with two portions of 20 ml ethyl acetate. The final aqueous layer was discarded and all the ethyl acetate layers collected. Then, the collected organic portion was evaporated at 90°C in a water bath until nearly dried (about 0.5 ml). The residue was dissolved with 5 ml 0.01 *M* HCl and made up to 50 ml with water. Samples were then taken for HPLC analysis.

3. Results and discussion

3.1. Selection of detection wavelength

Because the baseline noise was significant at wavelengths lower than 220 nm and a high background signal of sample solution occurred at wavelengths below 255 nm due to trace residuals of ethyl acetate, the present investigation was restricted to the range of 254–280 nm. Fig. 1 shows the relative detection sensitivity of sulfonamides under varied wavelength. As can be seen, a wavelength of 260 nm was satisfactory for all of the analytes, and was used throughout the studies.

3.2. Separation and identification of species

The sulfonamides and nicotinamide were separated in a reversed-phase ODS column and detected by UV detection under the conditions described in Section 2. A chromatogram of the sulfonamides is shown in Fig. 2. It is clear that all sulfonamide

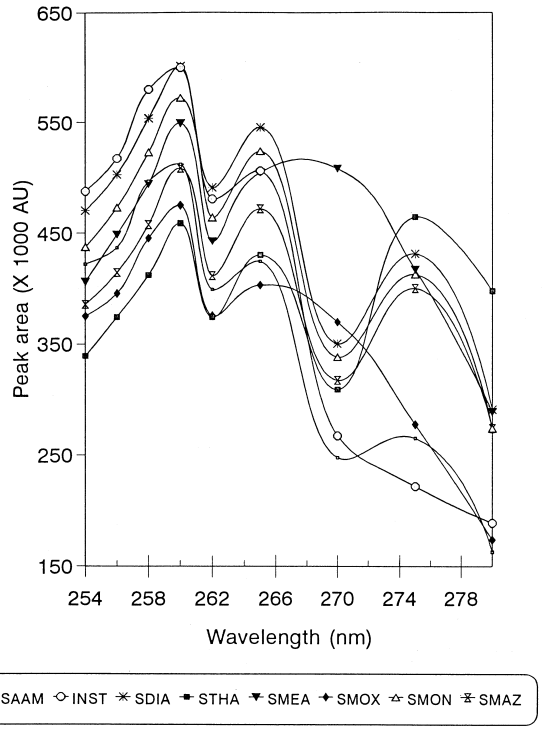

Fig. 1. Relative detection sensitivity of analytes under varied wavelengths. Detection of 1.25 μg/ml of each sulfonamide after HPLC separation.

species give sharp and symmetrical peaks. As shown in the chromatogram for standard species, the sulfonamides and nicotinamide are obviously well-separated.

3.3. Effect of buffer pH and concentration on capacity factor

The amphoteric character of sulfonamides reveals that the pH would bring about the changes in capacity factors and selectivity. Thus, the eluent should be carefully adjusted to an appropriate pH. Fig. 3 shows the influence of pH on the retention of sulfonamides. As can be seen, the retention time regularly varied significantly as the pH changes from 2.5 to 6.9. This can be explained by considering the pK_{a1} and pK_{a2} of each sulfonamide. If the pH is less than pK_{a1}, the sulfonamide is major in the structure of $H_3N^{(+)}–C_6H_4–SO_2–NH–R$, and when the pH is

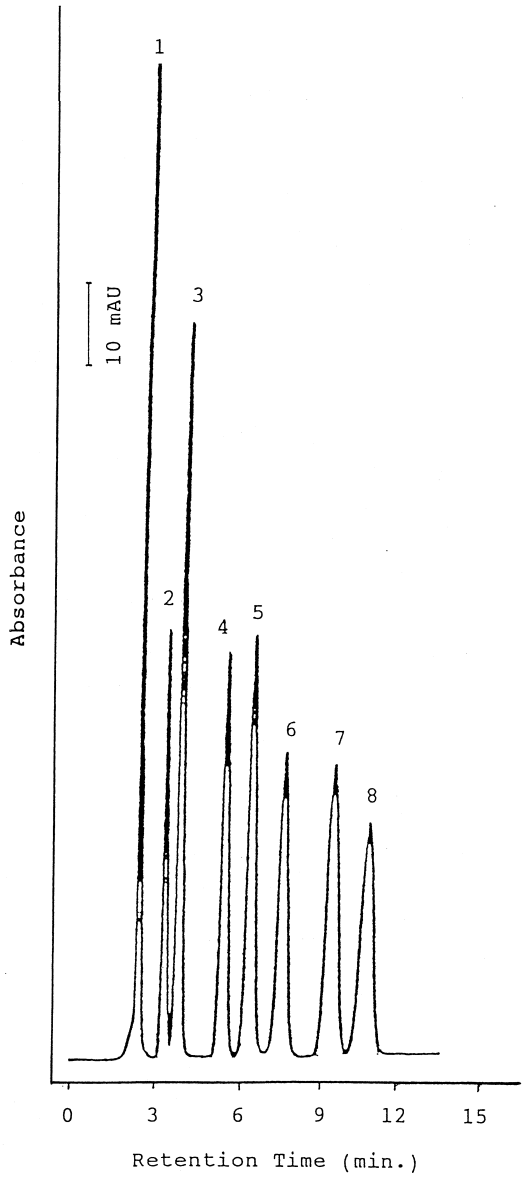

Fig. 2. Chromatograms of seven sulfonamides and nicotinamide. Chromatographic conditions: 20% methanol in 0.10 *M* pH 6.6 acetate buffer at 1.0 ml/min flow-rate. Sample injection: 800 μg/l each of sulfonamide, 20 μl. Peaks 1 to 8 are SAAM, nicotinamide, SDIA, STHA, SMEA, SMOX, SMON and SMAZ, respectively.

greater than pK_{a2}, $H_2N–C_6H_4–SO_2–N^{(-)}–R$ is the major form. Sulfonamides in these two forms are unfavorably retained on the reversed-phase ODS column. Thus, the retention time of a sulfonamide

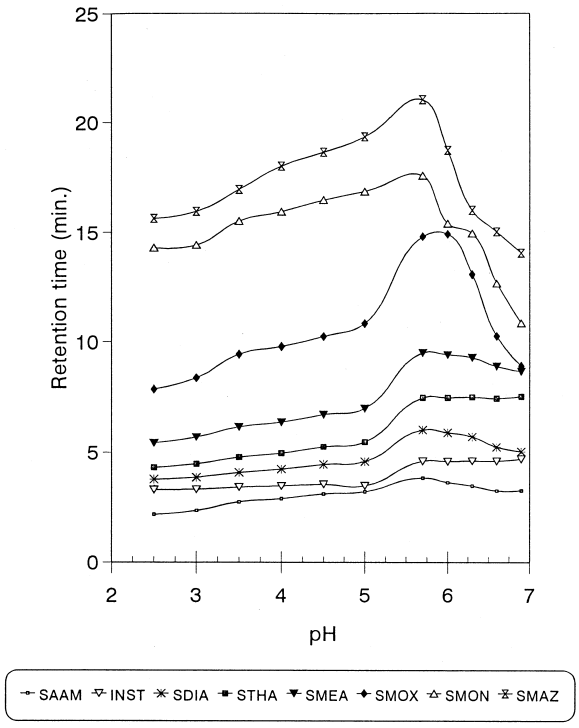

Fig. 3. The influences of pH on the sulfonamide retentions. Chromatographic conditions: 20% methanol in 0.10 *M* acetate buffer at various pH eluted at 1.0 ml/min flow-rate.

increased as the pH increased at lower pH, and then reached a maximum or level-off, followed by a decrease for the further-increase of pH. Overall, pH 6.6 elution gave an acceptable resolution. From our investigations, the acetate buffer rather than the phosphate buffer was selected to control the eluent pH due to its more stable baseline. In the study of buffer concentration effect, the observed peaks showed no significant changes in capacity factor when the separation was carried out in the range of 0.05–0.15 *M*, except SMOX and SMON could not be separated satisfactorily at 0.05 *M*. Therefore, 0.10 *M* acetate buffer at pH 6.6 was chosen as the eluent buffer.

3.4. Calibration graphs and detection limits

In order to test the applicability of the method for simultaneous determination of seven sulfonamides, calibration graphs were constructed over a concentration range of 50 μg/l to 10 μg/ml for all seven sulfonamides. The linear correlation coefficients are all above 0.9999. The reproducibility was examined with five replicate tests with the relative standard deviations (R.S.D.s) all being below 3.50%. The instrumental detection limits were calculated from the concentration of analyte that gives a signal equal to three times the peak-to-peak noise level of the baseline (blank injections) which are 4 μg/l for SAAM and SDIA, 10 μg/l for STHA and SMEA, 12 μg/l for SMOX, 15 μg/l for SMON and SMAZ.

3.5. Recoveries of sulfonamides from swine wastewater

Seven sulfonamides and nicotinamide (each 500 μg/l) were spiked with an adult-swine wastewater (free from sulfonamides). The pH was then adjusted to 6.6. After the ethyl acetate extraction and HPLC–UV determination, recoveries of sulfonamides were evaluated through a triplicate test. The recoveries are 86% for SAAM with 4.4% R.S.D., 98% for nicotinamide with 2.8% R.S.D., 99% for SDIA with 2.6% R.S.D., 97% for STHA with 1.6% R.S.D., 95% for SMEA with 2.6% R.S.D., 92% for SMOX with 3.0% R.S.D., 94% for SMON with 3.2% R.S.D. and 90% for SMAZ with 4.0% R.S.D. Theses results show that all sulfonamides can be recovered in acceptable levels.

3.6. Interference studies

Proteins, creatine and some organic acids were also present in the swine wastewater. Thus, protein, creatine, formic acid, acetic acid, propionic acid, *n*-butyric acid, pentanoic acid, *n*-caproic acid, enanthic acid and *n*-octanoic acid were added in the sample and eluted under the proposed chromatographic conditions. No serious interference occurred in the detection at 260 nm.

3.7. Quantitation of sulfonamides in swine wastewater

The applicability of this method to the determination of sulfonamides in real samples was tested by analyzing swine wastewater samples. Two samples of swine wastewater collected from adult-swine (>60 kg) and juvenile-swine (<60 kg) farms, were

analyzed by the proposed solvent extraction and HPLC–UV under the optimal experimental conditions. Fig. 4a and Fig. 4b demonstrate the chromatograms of these two samples, respectively. In Fig. 4b, it is clear that three sulfonamides existed in the juvenile-swine wastewater. The concentration of sulfonamides in the juvenile-swine wastewater were 76±1.9 μg/l, 77±2.3 μg/l and 69±5.6 μg/l (n=3) for SDIA, SMEA and SMOX, respectively, and in the adult-swine wastewater, no analytes were determined. It indicates that the juvenile-swine were under-treated with sulfonamides, and the owner of adult-swine farm seemed to follow the rule that only the juvenile-swine can be treated with sulfonamides.

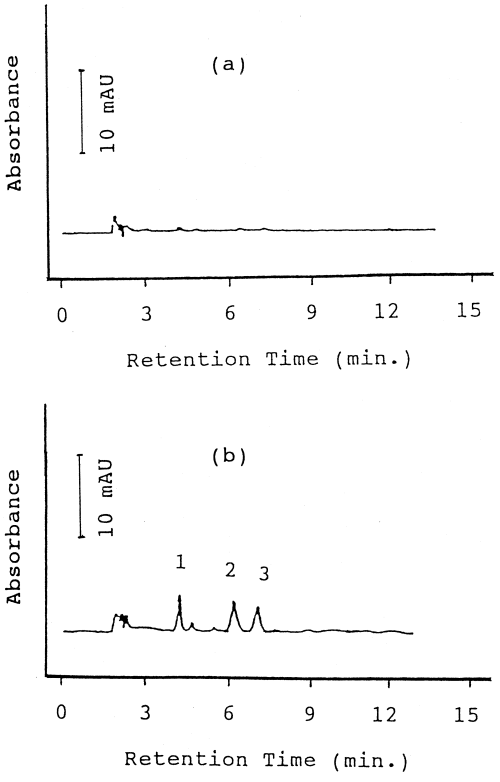

Fig. 4. Chromatograms of sulfonamides in swine wastewater (a) for adult-swine, (b) for juvenile-swine. Peaks 1 to 3 are SDIA, SMEA and SMOX, respectively.

4. Conclusions

This study demonstrates the applicability of a HPLC–UV method for the determination of seven sulfonamide residues in swine wastewater over the concentration range of 0.05 μg/ml to 10 μg/ml. The method can be applied for routine quantity evaluation and offers advantages in sensitivity, simplicity and reliability.

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