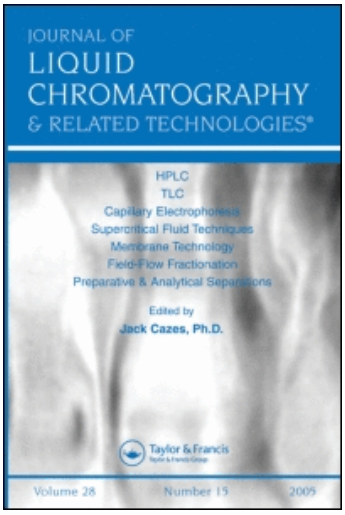

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Toxic/Harmful Solvents-Free Technique for HPLC Determination of Six Sulfonamides in Meat

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ABSTRACT

A simultaneous HPLC determination of six sulfonamides (SAs) [sulfadiazine (SDA), sulfadimidine (SDD), sulfamonomethoxine (SMM), sulfamethoxazole (SMX), sulfadimethoxine (SDM), and sulfaquinoxaline (SQ)] in meat (chicken, beef, and pork) is presented. The sample preparation is carried out by normal-phase matrix solid-phase dispersion (MPSD) with an aqueous ethanol solution. The HPLC determination is performed using a Mightysil RP-4 GP column and an isocratic mobile phase of 2% (v/v) acetic acid solution (pH 2.7, in water)–ethanol (75 : 25, v/v) with a photodiode array detector. Average recoveries spiked at 0.05–0.5 ppm for each drug are higher than 85% with standard deviations within 10%. In all the processes, no toxic/harmful solvents are used at all.

Key Words: HPLC; Sulfonamides; Meat; Toxic solvents.

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INTRODUCTION

Sulfonamides (SAs) (Fig. 1) are widely used for prevention or treatment of diseases in food-producing animals. Because of abuse such as an excessive administration and inappropriate withdrawal periods, there is a risk of drugs remaining in animal products. Sulfonamides residues could cause allergic or toxic, occasionally carcinogenic, reaction in consumers. In addition, widespread use of antibiotics may promote occurrence of antibiotic-resistant bacteria, whose infection is a serious threat. In order to eradicate misuse and prevent consumers from these problems, rigid inspection is essential. The maximum residue limit (MRL) in meat has been established for all SAs by the European Union (EU),[1] for sulfadimidine (SDD) by Codex Alimentarius Commission (CAC in FAO/WHO),[2] and Japan at 0.1 ppm.

Various analytical methods have been published for the determination of SAs in edible tissue.[3–7] One of them, matrix solid-phase dispersion (MSPD) developed by Barker,[8] improved some problems, numerous analytical steps, and high solvent consumption, with classic procedures. Matrix solid-phase dispersion has been applied to not only SAs but a wide range of analytes.[9] However, this method requires toxic solvents like hexane, methanol, acetonitrile, and dichloromethane, which highly pollute atmosphere, water, and ground (Table 1). Discharging of organic solvents contributes to the

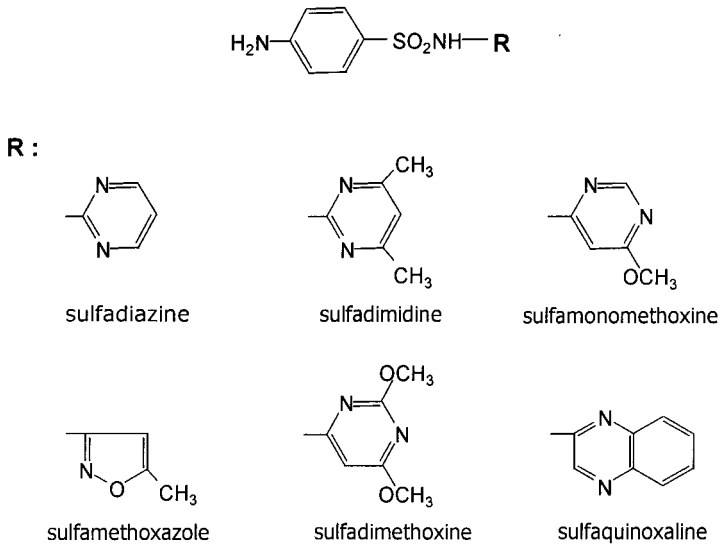

Figure 1. Structures of sulfonamides. R: substituent.

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Table 1. Hazard symbols and German Classification of Water Pollution Risk[a] for solvents concerning this study.

Solvent	Hazard symbol	Water pollution risk
Acetonitrile	Toxic	Water polluting substances
Acetic acid	Not toxic/harmful	Slightly water polluting substances
Dichloromethane	Harmful	Water polluting substance
Dimethylformamide	Toxic	Slightly water polluting substances
Ethanol	Not toxic/harmful	Slightly water polluting substances
Hexane	Harmful	Slightly water polluting substance
Methanol	Toxic	Slightly water polluting substances

[a]Ref.[14].

environmental pollution and is the international issue, and, also, their disposal is costly. The acceptable method must be rapid, precise, and economical in cost and time to permit the monitoring of a large number of samples, with negligible harm to the environment and analyst. Most importantly, the use of organic solvents should be made unnecessary wherever possible, and innocuous when used to ensure the environment and human health.[10–12] The authors have developed a normal-phase MSPD extraction for sulfadiazine (SDA), SDD, sulfamonomethoxine (SMM), sulfamethoxazole (SMX), sulfadimethoxine (SDM), and sulfaquinoxaline (SQ) in chicken using an aqueous ethanol solution.[13] Although, this technique has substantially reduced toxic solvents consumption, the determination with high-performance liquid chromatography (HPLC) required some toxic solvents, acetonitrile, and dimethylformamide.

The main goal is development of a toxic/harmful solvent-free method for the HPLC determination of the above SAs residues that is able to apply to beef and pork in addition to chicken. The sample preparation was performed according to the normal-phase MSPD technique established previously.[13]

EXPERIMENTAL

Materials and Reagents

Chicken, beef, and pork muscle tissues were purchased from local food markets and deep-frozen until analyses. Ethanol, distilled water, (HPLC grade), and acetic acid (analytical chemical grade) were obtained from

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Wako Pure Chem. Ltd. (Osaka, Japan). Alumina active neutral super I (activity super I, 70–200 mesh) (Alumina N-S) was obtained from ICN Biomedicals (Eschwege, Germany).

Six SA standards (SDA, SDD, SMM, SMX, SDM, and SQ) were obtained from Wako or Sigma Chemical (St. Louis, MO). Respective stock standard solutions of SAs were prepared by accurately weighing SDA, SDD, SMM, SMX, SDM, and SQ (10 mg) and dissolving in ethanol (100 mL). Working, mixed standard solutions of these six SAs were prepared by diluting the stock solutions with ethanol. These solutions can be kept at 4°C for up to 1 month.

Apparatus

The following apparatus were used for the sample preparation: rotary evaporator, Model EYELA N-N (Tokyo Rikakiki, Co., Tokyo, Japan); 0.45 μm disposable syringe filter unit, DISMIC-13_{HP} (hydrophilic PTFE) (ADVANTEC, Tokyo, Japan).

Mightysil RP-4 GP (250 × 4.6 mm I.D.) with a guard column (5 × 4.6 mm I.D.) were obtained from Kanto Chemical Co. (Tokyo, Japan).

Analyses of standard and extracted SAs were conducted using a Jasco HPLC system (Model PU-980 pump and DG-980-50 degasser) (Jasco, Tokyo, Japan) equipped with an SPD-$M10A_{vp}$ diode array detector (Shimadzu, Kyoto, Japan), interfaced with Fujitsu FMV-5133D7 personal computer (Fujitsu, Tokyo, Japan).

The separation was performed on a Mightysil RP-4 GP using a mixture of 2% acetic acid solution (pH 2.7, in water)–ethanol (75 : 25, v/v) as the mobile phase at a flow-rate of 0.8 mL/min at an ambient temperature. The monitoring wavelength was set at 267 nm, which was an average maximum absorbance for six SAs.

Procedure

An aliquot (0.3 g for beef, 0.5 g for pork and chicken) of the samples was blended with 2 g of Alumina N-S to obtain a homogeneous mixture. The mixture was transferred to a syringe barrel and eluted with 10 mL of a 70% (v/v) aqueous ethanol solution by gravity flow. The eluate was evaporated to dryness, and the residue was dissolved in 1 mL of the HPLC mobile phase. The solution was filtered through a 0.45 μm filter unit. Ten microliter of the filtrate was injected into the present HPLC system.

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RESULTS AND DISCUSSION

HPLC Operating Conditions

Acetonitrile and methanol are usually used in the mobile phase for reversed-phase (RP) HPLC separation of various compounds. In a previous paper for the MSPD method,[13] we also have used acetonitrile and dimethylformamide as the mobile phase. According to Hazard Symbols in Merck catalogue, acetonitrile, methanol, and dimethylformamide are ranked as toxic, the influence of ethanol on the environment and human is negligible. Moreover, German Classification of Water Pollution Risk ranks acetonitrile as higher water polluting substances than ethanol (Table 1).[14] In order to minimize hazardous influence to the environment and human beings, analyses should be performed without these toxic solvents. We have previously reported the acceptable HPLC determination of seven SAs in milk using Mightysil RP-4 GP (C_4) and a 25% aqueous ethanol solution.[15] The same HPLC condition could not apply to this study, because unlike milk, meat is a complex matrix containing much interference like lipid and protein. By adding a little acetic acid (not toxic/harmful) to the mobile phase, the target compounds and interference could be separated completely.

Figure 2 shows representative HPLC chromatograms obtained from meat samples. The target compounds were free from interference and detected within 14 min. The present method did not require gradient elution and any toxic/harmful solvents to achieve the separation.

Recoveries and Calibration

Recovery study for validation of the method was carried out at four different spiking levels (0.05, 0.1, 0.2, and 0.5 ppm for each drug). Table 2 shows average recoveries, their standard deviations (SDs), interassay variabilities, and limits of quantitation (LOQs). Recoveries were calculated by making a comparison between extracts and standards for peak height. These values well fulfill the acceptable criteria shown in Table 3 that Codex sets up for the residual analysis.[16] Limits of quantitation was defined as the average background of sample plus 10 times the SD of standard. The LOQs for six SAs ranged from 0.006 to 0.04 ppm. These limits were valid for practical use. The calibration graphs were drawn by a standard addition method and composed of four points ranging from 0.05 to 0.5 ppm. The correlation coefficient (r) for each SA was >0.999 ($P < 0.01$).

A photodiode array detector is the confirmatory tool, which can identify target compounds by both retention times and absorption spectra. Since the

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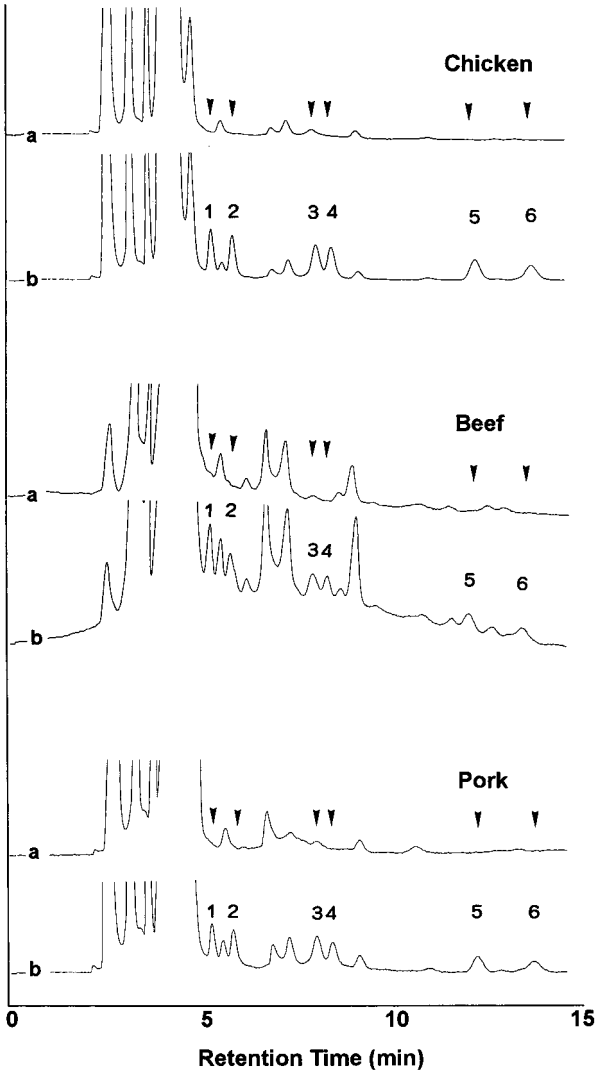

Figure 2. Representative HPLC chromatograms obtained from meat samples (photodiode array detector set at 267 nm): (a) blank; (b) fortified (0.1 ppm for each drug). Peaks: 1 = sulfadiazine; 2 = sulfadimidine; 3 = sulfamonomethoxine; 4 = sulfamethoxazole; 5 = sulfadimethoxine; 6 = sulfaquinoxaline.

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Table 2. Validation of the present method.

Fortification level (ppm)	Recovery (%) (mean ± SD, $n = 5$)					
	SDA	SDD	SMM	SMX	SDM	SQ
			Chicken			
0.05	94.6 ± 2.9	93.7 ± 3.4	92.1 ± 2.2	95.6 ± 3.4	91.6 ± 1.6	95.0 ± 3.5
0.1	96.5 ± 2.6	92.2 ± 1.3	95.2 ± 2.7	94.3 ± 2.0	92.0 ± 5.3	91.6 ± 3.7
0.2	95.7 ± 3.4	93.2 ± 3.2	95.0 ± 2.7	94.2 ± 3.1	96.0 ± 4.0	97.6 ± 3.0
0.5	94.7 ± 3.2	93.9 ± 3.9	93.6 ± 2.0	94.8 ± 3.2	94.7 ± 2.9	96.0 ± 4.4
Interassay variability (% ±SD)	3.0 ± 0.4	3.0 ± 1.1	2.4 ± 0.4	2.9 ± 0.6	3.5 ± 1.6	3.7 ± 0.6
LOQ[a] (ppm)	0.006	0.010	0.014	0.015	0.026	0.033
			Beef			
0.05	92.4 ± 8.0	96.4 ± 8.2	98.4 ± 9.9	97.1 ± 8.8	95.2 ± 9.4	95.5 ± 8.1
0.1	95.7 ± 8.3	95.0 ± 9.4	94.6 ± 7.5	90.2 ± 6.1	99.4 ± 5.0	92.4 ± 7.2
0.2	100.8 ± 6.9	93.2 ± 6.1	92.1 ± 5.9	92.4 ± 9.6	97.3 ± 9.4	92.7 ± 7.1
0.5	95.1 ± 6.3	100.7 ± 7.0	99.1 ± 6.0	98.9 ± 4.4	98.3 ± 9.5	95.8 ± 5.9
Interassay variability (% ±SD)	7.7 ± 1.2	8.0 ± 1.5	7.6 ± 1.8	7.7 ± 2.6	8.6 ± 2.4	7.6 ± 1.0
LOQ[a] (ppm)	0.007	0.009	0.015	0.014	0.026	0.031
			Pork			
0.05	90.5 ± 7.5	90.5 ± 6.9	96.4 ± 8.1	85.4 ± 5.0	94.0 ± 5.5	95.2 ± 5.2
0.1	88.4 ± 4.3	91.4 ± 4.3	92.9 ± 3.6	92.5 ± 3.8	94.5 ± 5.0	94.0 ± 4.2
0.2	95.5 ± 5.1	98.5 ± 4.2	95.4 ± 3.5	98.2 ± 5.3	98.2 ± 7.3	95.5 ± 3.7
0.5	94.3 ± 3.6	94.6 ± 3.0	93.9 ± 2.1	95.1 ± 2.4	92.7 ± 2.2	92.5 ± 3.8
Interassay variability (% ±SD)	5.6 ± 1.9	5.0 ± 1.9	4.6 ± 2.7	4.5 ± 1.5	5.3 ± 2.1	4.5 ± 0.7
LOQ[a] (ppm)	0.008	0.010	0.015	0.014	0.027	0.032

[a]LOQ = limit of quantitation.

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Table 3. Acceptable criteria of Codex Alimentarius Commission[a] for residual analysis.

MRL (ppm)	$\geq 0.001 \leq 0.01$	$\geq 0.01 \leq 0.1$	≥ 0.1
Recovery (%)	$60 \sim 120$	$70 \sim 110$	$80 \sim 110$
Coefficient of variation (%)	<30	<20	<15

[a]Ref.[16].

extracts were identical with respective SA standards for two pieces of information, they were identified as SAs.

CONCLUSION

Rigid inspection to prevent consumers from drug residues is needed to analyze a large number of samples, that is, it requires a great volume of solvents. A decrease in toxic/harmful solvents consumption is a positive direction for analytical science, because environmental problems are a world-wide concern. The proposed toxic/harmful solvents-free method, normal-phase MSPD followed by HPLC, achieved the simultaneous determination of six SAs in meat with high accuracy and confirmation. Therefore, this method can be suitable for routine technique in laboratories.

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