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Short communication

Application of shielded column liquid chromatography for determination of sulfamonomethoxine, sulfadimethoxine, and their N_4-acetyl metabolites in milk

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Abstract

A hazardous-chemical free method for simultaneous determination of sulfamonomethoxine (SMM), sulfadimethoxine (SDM), and their N_4-acetyl metabolites in raw milk using shielded column liquid chromatography is developed. The target analytes are extracted by mixing with ethanol–acetic acid (97:3, v/v) followed by centrifugation. The procedure uses a Hisep shielded hydrophobic phase (SHP) column, isocratic elution with 0.1% acetic acid solution (pH 3.1, in water)–ethanol (75:25, v/v), and a photo-diode array detector. Average recoveries from samples spiked at 25–500 ng/ml for each drug were >81% with relative standard deviations within 5%. The limits of quantitation were <25 ng/ml.

Keywords: Sulfamonomethoxine; Sulfadimethoxine

1. Introduction

Sulfamonomethoxine (SMM) and sulfadimethoxine (SDM) are frequently used for prevention or treatment of diseases to food-producing animals in Japan and all the countries of the world, respectively. There is a risk of drugs remaining in animal products because of illegal use such as an excessive administration and an inappropriate withdrawal period. Drug residues to consumers and promote occurrence of antibiotic-resistant bacteria.

Sulfonamides, including SMM and SDM, can be acetylated at the N_4-position. The acetylated sulfonamides are of particular concern as they are appreciably less soluble in water than the parent drugs and the renal toxicity of sulfonamides has been attributed to precipitation of these conjugations in kidney [1]. It is, therefore, important to detect both the parent drugs and the N_4-acetyl (Ac) metabolites (Fig. 1) in animal products.

Overuse of SMM or SDM in cows is of great concern because drug residues and their Ac-metabolite are found in milk. Residue monitoring of the above compounds in milk is therefore an important activity to guarantee the safety of food. The method should be rapid, easy to perform, and inexpensive. Previous analytical methods [2–13] have described that sulfonamides and/or Ac-metabolites residues in animal products, including milk, could be acceptably determined by high performance liquid chromatography (HPLC). However, these methods have some disadvantages. Procedures require the use of some hazardous solvents, like acetonitrile [2–6,12,13], dichloromethane [2–6], and hexane [2–5], as the extraction solvents or the HPLC mobile phase and some hazardous reagents, like trichloroacetic acid [10] and perchloric acid [9,11], as the extraction and deproteination in the sample preparations. These substances are widely used and highly valued because of their excellent solvency in a wide range of analytes.

In this paper, we have developed a simultaneous LC determination of SMM, SDM, and their Ac-metabolites in raw milk without the use of complex extraction/clean-up techniques. To this end, the present study a applied shielded column LC.

2. Experimental

2.1. Materials and reagents

Raw milk served as a sample, it was stored in a refrigerator until analysis.

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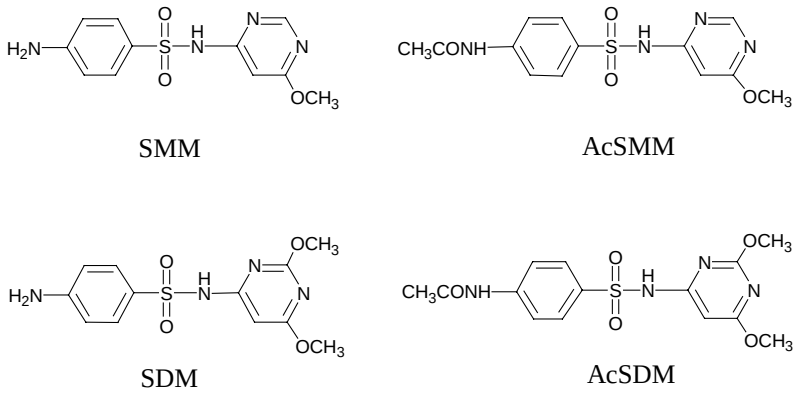

Fig. 1. Structures of target compounds.

SMM, SDM, ethanol, distilled water (HPLC grade), and acetic acid (analytical chemical grade) were obtained from Wako Pure Chem. (Osaka, Japan). AcSMM and AcSDM were generous gifts from Dr. Miura (Daiichi Seiyaku, Tokyo).

Each stock standard solution was prepared by accurately weighing (10 mg) and dissolving it in ethanol (100 ml). Working mixed standard solutions were prepared by diluting the stock solutions with distilled water. These solutions can be kept at 4 °C for up to 1 month.

2.2. Procedure

A 0.3 ml sample was placed into a microcentrifuge tube together with 0.1 ml of ethanol–acetic acid (97:3, v/v) and homogenized with a handheld ultrasonic homogenizer (Model HOM-100, 2 mm i.d. chip, Iwaki Glass Co. Ltd., Funabashi, Japan) for 30 s. The tube was centrifuged at 10,000 × *g* for 5 min at 4 °C with a microcentrifuge (Biofuge Fresco, Kendo Lab. Products, Hanau, Germany). The supernatant was injected directly on the LC column. LC analyses were carried out using a LC-10ADvp system equipped with an SPD-M10Avp photo-diode array detector (Shimadzu, Kyoto, Japan). The analytical column was a Hisep shielded hydrophobic phase (SHP) column (250 mm × 4.6 mm i.d., 5 μm) (Supelco, Bellefonte, PA, USA). The separation was performed using 0.1% (v/v) acetic acid solution (pH 3.1, in water)–ethanol (75:25, v/v) as the mobile phase at a flow rate of 1.2 ml/min at 40 °C. The injection volume was 20 μl.

3. Results and discussion

3.1. Sample preparation

The addition of 3% acetic acid in the extraction solvent was effective to prevent emulsion formation. After centrifugation, the target compounds were recovered aqueous layer. No further purification prior to LC analysis was needed (see Fig. 2). The time required for sample preparation was less than 10 min.

3.2. LC optimal conditions

The shielded column has the advantage of enabling rapid separation of the target compounds (low molecular weight compounds) and the extractable proteins (high molecular weight compounds) [12–14]. The sample preparation gets finished with rapid single step like the present procedure.

Uno et al. have previously reported acceptable determination of SMM and AcSMM from animal and fish serum by LC using a Hisep-SHP column (150 mm × 4.6 mm i.d.) (the short column) and acetonitrile solution as the mobile phase

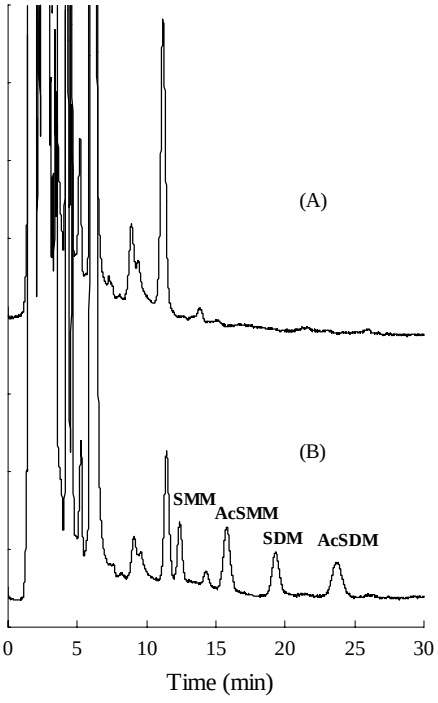

Fig. 2. UVLC chromatograms obtained for (A) blank and (B) fortified (50 ng/ml of each drug) milk samples.

Table 1
Recoveries and R.S.D.s of target compounds from milk

Fortification level (ng/ml)	Recovery (%) (mean, $n = 5$)			
	SMM	AcSMM	SDM	AcSDM
25	85 (1.1)	87 (2.2)	81 (1.8)	87 (2.3)
50	87 (2.7)	84 (1.9)	83 (1.6)	90 (4.5)
100	84 (1.9)	86 (2.2)	84 (2.5)	84 (1.8)
500	84 (1.8)	86 (1.3)	82 (3.8)	85 (3.1)
Correlation coefficient[a]	0.999	0.999	0.999	0.998
LOQ[b] (ng/ml)	9	10	13	25

[a] Constructed from four points and each point represented the mean of five injections.
[b] LOQ was defined as the average background of samples (fluctuations of the baseline) plus ten times the standard deviation (S.D.).

[12]. In this case a longer column (250 mm × 4.6 mm i.d.) was needed to optimize the separation.

The chromatogram with complete separation of all the target compounds, their sharp peaks, and their short retention times was obtained by the long Hisep-SHP column (250 mm × 4.6 mm i.d.) and an isocratic mobile phase of 0.1% acetic acid solution (pH 3.1, in water)–ethanol (75:25, v/v). The monitoring wavelength was set at 267 nm because an average maximum absorption spectrum for SMM, SDM, AcSMM, and AcSDM dissolved in the mobile phase was 267 nm. Under the present conditions, oxytetracycline and sulfadimidine, which are frequently used in food-producing animals worldwide, did not interfere with the target compounds.

Fig. 2 shows LC chromatograms for obtained for milk samples. The extracts were free from interfering compounds for quantitation and identification in all HPLC chromatograms. LC combined with photo-diode array detection confirmed the peak identities. The total time required for the analysis of one sample was less than 40 min.

The present HPLC system did not cause column clogging, peak broadening, and variation of retention times throughout analyses over 500 sample injections without 'pre-column washing' after an analysis.

In our previous paper for determination of seven sulfonamides, bar out their Ac-metabolites, in milk without using hazardous-chemicals [15], the sample preparation was doubly carried out by homogenizing extraction with ethanol solution followed by deproteination using an ultra-filtration unit. The present procedure did not need ultra-filtrating treatment before the shielded column LC and was achieved with considerable saving of analytical time and cost. In addition, compared with our other previous methods using perchloric acid or trichloroacetic acid [9–11] this is another environmental friendly procedure.

3.3. Method validation and application

Table 1 shows the average recoveries from milk samples with their relative standard deviations (R.S.D.s) and limits of quantitation (LOQs). The recoveries and R.S.D.s meet the criteria for residue analysis of the Codex (recovery 70–110% and R.S.D. < 20%, for MRL < 100 ng/ml) [16]. In a practical analysis for the residue monitoring, the LOQs for the four target compounds ranged from 9 to 25 ng/ml. These LOQs were well below the maximum residue limit (MRL) (100 ng/ml) established by European Union [17]. We generated the spiked recovery graph as the practical calibration line by plotting peak heights of fortified sample extracts ranging from 25 to 500 ng/ml. The resulting correlation coefficient (r) for each compound was >0.998 ($P < 0.01$). As an application, 20 samples of commercial raw milk purchased in Osaka were analyzed using the present method. No samples contained detectable concentrations of SMM, SDM, and their Ac-metabolites. All chromatograms were free from interferences.

4. Conclusions

The proposed shielded column LC method enabled the simultaneous determination of SMM, SDM, AcSMM, and AcSDM with the following advantages: (1) using a hand-held ultrasonic homogenizer makes the sample preparation especially easy and effective; (2) the procedure has an analysis time of less than 40 min/sample; (3) the procedure is economical, and allows reproducible recoveries and using no hazardous-chemicals. Therefore, this method is useful for practical residue monitoring and studying pharmacokinetics of SMM, SDM, and their Ac-metabolites in milk.

References

[1] T.B. Vree, Y.A. Hekster, M.W. Tijhuis, Antibiot. Chemother. 35 (1985) 66.
[2] S.L. Boulaire, J.-C. Bauduret, F. Andre, J. Agric. Food Chem. 45 (1997) 2134.
[3] L.V. Walker, J.R. Walsh, J.J. Webber, J. Chromatogr. 595 (1992) 179.
[4] A.R. Long, L.C. Hsieh, M.S. Malbrough, C.R. Short, S.A. Barker, J. Assoc. Off. Anal. Chem. 73 (1990) 868.
[5] A.R. Long, L.C. Hsieh, M.S. Malbrough, C.R. Short, S.A. Barker, J. Agric. Food Chem. 38 (1990) 423.
[6] G. Stoev, A. Michailova, J. Chromatogr. A 871 (2000) 37.
[7] R.J. Maxwell, A.R. Lightfield, J. Chromatogr. B 715 (1998) 431.
[8] N. Furusawa, J. Chromatogr. Sci. 40 (2002) 355.
[9] N. Furusawa, Anal. Chim. Acta 481 (2003) 255.
[10] N. Furusawa, LC GC North Am. 21 (2003) 362.
[11] N. Furusawa, Chromatographia 57 (2003) 317.
[12] K. Uno, I. Maeda, J. Chromatogr. B 663 (1995) 177.
[13] R. Ueno, T. Aoki, J. Chromatogr. B 682 (1996) 179.
[14] V.K. Agarwal, J. Liquid Chromatogr. 13 (1990) 2475.
[15] N. Furusawa, K. Kishida, Fresenius J. Anal. Chem. 371 (2001) 1031.
[16] Codex Alimentarius Commission, Joint FAO/WHO Food Standards Program, Residues of Veterinary Drugs in Food, vol. 3, Second ed., Rome, Italy, 1993.
[17] Commission of the European Communities, Establishment by the European Community of Maximum Residue Limits (MRLs) for Residues of Veterinary Medical Products in Foodstuffs of Animal Origin, The Rules Governing Medical Products in European Community VI, October 1991, ECSC-EEC-EAEC, Brussels, Belgium.