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Quantitation and confirmation of six sulphonamides in meat by liquid chromatography–mass spectrometry with photodiode array detection

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Abstract

Multiresidue analysis of six sulphonamides (SAs) (sulphadiazine, sulphadimidine, sulphamonomethoxine, sulphamethoxazole, sulphadimethoxine, and sulphaquinoxaline) in meat (beef, pork, and chicken) using liquid chromatography (LC)–mass spectrometry (MS) with photodiode array (PDA) detection is presented. The sample preparation is carried out by normal-phase matrix solid-phase dispersion (MSPD) with an ethanol solution. The LC–MS determination is performed using a Mightysil RP-4 GP column and an isocratic mobile phase of 0.3% (v/v) acetic acid solution (pH 3.4, in water)–ethanol (83:17, v/v) with an atmospheric pressure chemical ionization (APCI) MS on positive-ion mode. Average recoveries spiked at 0.05–0.5 ppm for each drug were higher than 90% with relative standard deviations between 1% and 6%. In all the processes, no toxic solvents were used at all.

Keywords: Liquid chromatography; Mass spectrometry; Sulphonamides

1. Introduction

Sulphonamides (SAs) are widely used in food-producing animals for prevention or treatment of diseases and growth-promoting purposes. There is a risk of SA residues remaining in animal products if these drugs have been improperly administered or if the withdrawal period has not been observed. Drug residues may cause allergic or toxic reaction to consumers and promote occurrence of antibiotic-resistant bacteria. In addition, sulphadimidine (SDD) mainly applied to swine is suspected of potential carcinogen (NCTR Technical Report Experiment No. 418, 1988). The European Union (EU) has set a maximum residue limit (MRL, 0.1 ppm) for SAs in foods of animal origin such as meat, milk, and eggs (Commission of the European Communities, 1991).

EU Commission Decision 93/256/EEC states that "Methods based only on chromatographic analysis without the use of molecular spectrometric detection are not suitable for use as confirmatory methods". Liquid chromatography (LC)–mass spectrometry (MS) is the very ideal technique to detect nonvolatile, polar compounds such as SAs.

Several approaches involving LC–MS or LC–MS/MS have been reported for determination of SAs in animal products (Baere et al., 2000; Cavaliere, Curini, Corcia, Nazzari, & Samperi, 2003; Combs, Ashraf-Khorassani, & Taylor, 1999; Doerge, Bajic, & Lowes, 1993; Dost, Jones, & Davidson, 2000; Fuh & Chan, 2001; Heller et al., 2002; Ito et al., 2000; Rhijin, Lasaroms, Berendsen, & Brinkman, 2002; Volmer, 1996). However, these methods have the following problems:

- The extraction and clean-up involves varying analytical steps that are labor and time consuming.
- Recoveries are sometimes low and variable.
- The sample preparation or determination is not economical in cost.
- A large quantity of toxic solvents such as acetonitrile, methanol, and methylene chloride are used as extracting

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solvents and LC mobile phases, which can be harmful to the environment.

The author has previously developed a rapid and simple method for six SAs in meat using normal-phase matrix solid-phase dispersion (MSPD) followed by high-performance liquid chromatography (HPLC) (Kishida & Furusawa, 2001; Kishida & Furusawa, 2003). Although this technique has reduced analytical time, cost, and toxic solvent consumption, the determination with HPLC was not suitable as a confirmatory method.

This paper presents the quantitation and confirmation of six SAs in meat (beef, pork, and chicken) using LC-MS and photodiode array (PDA) detection without use of toxic solvents.

2. Experimental

2.1. Materials and reagents

Beef, pork, and chicken 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 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 (sulphadiazine (SDA), sulphadimidine (SDD), sulphamonomethoxine (SMM), sulphamethoxazole (SMX), sulphadimethoxine (SDM), and sulphaquinoxaline (SQ)) were obtained from Wako or Sigma Chemical (St. Louis, MO, USA). 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 and stable for up to one month.

2.2. Apparatus

The following apparatus were used for sample preparation: homogenizer, Model NS-50 (Microtec Co., Chiba, Japan); 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).

Two silica-based reversed-phase columns: LiChrospher RP-8 and Mightysil RP-4 GP (250 × 4.6 mm I.D.) with their guard columns (5 × 4.6 mm I.D.) were obtained from Merck (Darmstadt, Germany) or Kanto Chemical Co. (Tokyo, Japan).

Analyses were carried out using a LCMS-2010 system equipped with an SPD-$M10A_{vp}$ PDA detector with an atmospheric pressure chemical ionization (APCI) interface (Shimadzu, Kyoto, Japan). The present analytical conditions for LC-MS are listed in Table 1.

Table 1
LC-MS operating conditions

Analytical column	: Mightysil RP-4 GP
Mobile phase	: 0.3% (v/v) acetic acid solution (pH 3.4, in water)–ethanol (83:17, v/v)
Flow rate	: 1.2 ml/min
Column temperature	: 28 °C
Monitoring wavelength	: 267 nm
Injection volume	10 μl
Probe voltage	: +4.5 kV (APCI-positive-ion mode)
Probe temperature	: 400 °C
Nebulizing gas flow	: 2.5 l/min (N_2)
Selected ion monitoring	: SDA (*m/z* 251.3), SDD (*m/z* 279.3), SMM (*m/z* 281.3) SMX (*m/z* 254.3), SDM (*m/z* 311.3), SQ (*m/z* 301.3)

2.3. Procedure

An accurate 0.5 g 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. The eluate was evaporated to dryness, and the residue was dissolved in 1 ml of the mobile phase. The solution was filtered through a 0.45 μm filter unit. The filtrate was analyzed by the present LC-MS system.

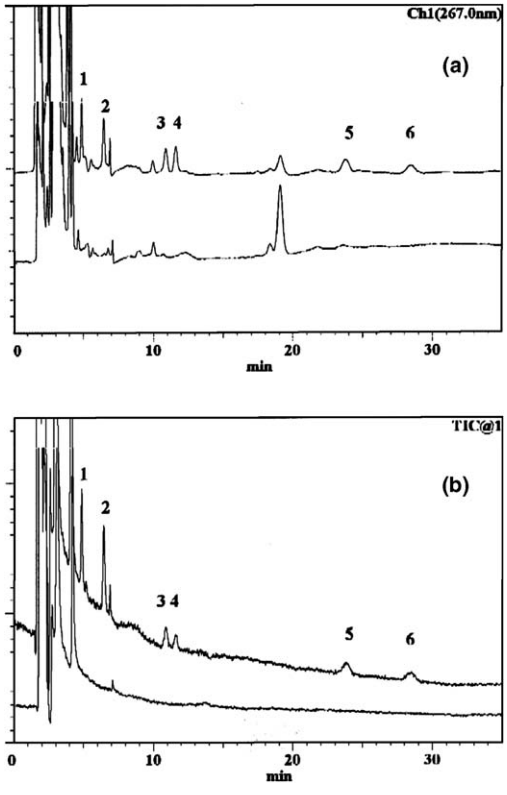

Fig. 1. (a) HPLC and (b) TIC chromatograms of fortified (0.1 ppm for each drug) (top) and blank (bottom) beef samples. Peaks: 1 = SDA, 2 = SDD, 3 = SMM, 4 = SMX, 5 = SDM, 6 = SQ.