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Analytical Methods

Magnetic solid phase extraction followed by high-performance liquid chromatography for the determination of sulphonamides in milk samples

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ABSTRACT

A simple and effective method based on magnetic solid-phase extraction combined with high performance liquid chromatography was used for the determination of nine sulphonamides in milk samples. The extraction and cleanup via silica-based magnetic adsorbent dispersion in milk samples followed by the magnetic isolation and desorption of the analytes using NaOH–methanol. Three different magnetic phenyl silica adsorbents were synthesized by varying the molar ratio of phenyltrimethylsilane and tetramethylorthosilicate; these adsorbents were evaluated for sulphonamides retention in terms of their pH and degree of hydrophobicity. The optimal conditions were a pH of 6.0 and a magnetic:sorbent ratio of 2:1. Under optimal conditions, limits of detection ranging from 7 to 14 μg L^{-1} were obtained. The method was validated according with the European Commision Decision 2002/657/EC. The proposed method was applied to analyse sulphonamides in 27 milk samples of different brands. Thirteen samples tested were positive for the presence of sulphonamides.

1. Introduction

Sulphonamides (SAs) are a group of synthetic antimicrobials that are frequently employed for clinical and veterinary purposes to control bacterial infection. These antimicrobials are used for their broad antibacterial spectrum, high efficacy, and low cost (Wang et al., 2012). A variety of SAs are available. However, the SAs most frequently employed in veterinary medicine are sulfadiazine (SDZ), sulfathiazole (STZ), sulfamethazine (SMZ), sulfamethoxypyridazine (SMPZ), sulfachloropyridazine (SCP), sulfamethoxazole (SMX), sulfisoxazole (SFX), sulfadimethoxine (SDM) and sulfaquinoxaline (SQX) (Korpimaki et al., 2004; Wang et al., 2007, 2012).

In animal husbandry, SAs are directly administered or added to the feed of poultry, pigs, and cattle to prevent and treat gastrointestinal and respiratory diseases. Additionally, SAs are used in some countries as growth promoters for food-producing animals (Pereira et al., 2012; Wang et al., 2012). The uncontrolled use of sulphonamides can lead to the accumulation of these drugs in animal tissues. The presence of these residues, despite their minimal amounts, can induce adverse effects in humans, such as allergic reactions in hypersensitive individuals. Another long-term effect may be carcinogenicity, and prolonged exposure can result in the selection of resistant bacteria in the human body (Di Corcia & Nazzari, 2002; Koesukwiwat, Jayanta, & Leepipatpiboon, 2007; Zayas-Blanco, García-Falcón, & Simal-Gándara, 2004). In dairy cows, SAs are applied in significant amounts and are excreted in the milk. To ensure safety for consumers, the European Union (EU) commission and Drug Administration (FDA) has established a maximum reside limit (MRL) of 100 μg L^{-1} and 100 μg kg^{-1}, respectively for SAs in food of animal origin, such as milk (Commission Regulation (EC) No. 281/96, 1999; Commission Regulation (EEC) No. 2377/90, 1990; Koesukwiwat et al., 2007; Wenjun, Chunming, & Minglin, 2011).

A variety of analytical methodologies have been developed and reported for the determination of SA residues in foodstuffs at the μg kg^{-1} or μg L^{-1} levels. These methods, used individually or sequentially according to the complexity of the analytical matrix, include immunoassay (Keizer, Bienenmann-Ploum, Bergwerff, & Haasnoot, 2008), capillary electrophoresis (Ming-Ren & Su-Yi, 2003; Soto-Chinchilla, García-Campaña, Gámiz-Gracia, & Cruces-Blanco, 2004), gas chromatography (Reeves, 1999), high

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performance liquid chromatography/mass spectrometry (HPLC/MS) (Bogialli, Ascenzo, Di Corcia, Laganà, & Nicolardi, 2008; Di Corcia & Nazzari, 2002), and high performance liquid chromatography (HPLC) (Hyun-Hee, Jung-Bin, Yun-Hee, & Kwang-Geun, 2009; Zayas-Blanco et al., 2004). However, some techniques can be expensive, and many laboratories do not have access to this instrumentation, especially in developing countries. Consequently, the lack of residue control in animal-origin foods for developing nations is a serious handicap that impedes the exportation of these products to countries in which MRLs are established. In this way, HPLC has become a useful technique for analysing antibiotics because it offers high separation efficiency and short analysis times. However, this technique is sometimes insufficient for the direct determination of such antibiotic residues. Trace SA residues must be preconcentrated in food samples such as milk, so they can be detected by HPLC.

One of the most difficult stages in the analysis of antibiotics is the extraction, cleanup, and preconcentration of the samples. Solid phase extraction (SPE) is currently the most commonly method employed for simultaneous extraction and cleanup. However, the SPE procedure is expensive and sometimes labour-intensive (Zayas-Blanco et al., 2004). For SAs extraction, the sorbent selection depends on several factors. Common adsorbents are silica-based (C8, C18) (Heller et al., 2002; Reeves, 1999). Recently, adsorbents combined with different types of polymers, such as hydrophilic–lipophilic balanced (HLB) polymers, have been used as an alternative to chromatographic separations, and, in some cases, these combined adsorbents were found to be more selective during the separation of different compounds (Ming-Ren & Su-Yi, 2003).

Magnetic solid phase extraction (MSPE) has received considerable attention in recent years due to its potential applications to cell isolation, enzyme immobilisation, protein separation and the preconcentration of organic compounds (Meng et al., 2011; Safarik & Safarikova, 1999). This technique is based on the dispersion of a magnetic adsorbent in the liquid sample. The magnetic adsorbents with analytes adsorbed on the surface can be isolated and eluted with an appropriate solvent. The most attractive property of MSPE is the easy isolation of the magnetic adsorbents from sample solutions by applying an external magnetic field, which minimises the sample treatment time. MSPE has allowed the selective separation of organic compounds, including antibiotics, anti-inflammatory drugs, pesticides and phenolic compounds that are present in different matrices (Aguilar-Arteaga, Rodriguez, Miranda, Medina, & Barrado, 2010; Ibarra, Rodriguez, Paez-Hernandez, Santos, & Miranda, 2012).

In this work, a new method for analysing the SAs in milk samples was developed to analyse whole milk samples according to the EU MRLs. This method involves sample pretreatment by MSPE using a paramagnetic adsorbent. The solids were magnetite-embedded with silica functionalized with phenyl chains. The magnetic solids were synthesized, characterised and employed in the cleanup and preconcentration during SAs determination. The magnetic solids are selective and easier to use than classic methodologies, such as SPE.

2. Experimental

2.1. Reagents and chemicals

All solutions were prepared by dissolving the respective analytical grade reagent in deionized water with a resistivity not less than 18.0 MΩ cm, provided by a Milli-Q system (Millipore, Bedford, MA, USA). Sodium hydroxide and acetic acid were obtained from J.T. Baker (Phillipsburg, NJ, USA). Methanol was supplied from Sigma–Aldrich (Taufkirchen, Germany). The mobile phases used for HPLC experiments consisted of two solutions, one a solution of acetonitrile with 1% formic acid, and the second a solution of water with 1% formic acid. Both solutions were filtered through a 0.45-μm filter prior to use.

Sulfachloropyridazine (98%), sulfadiazine (99%), sulfadimethoxine (99%), sulfamethazine (99%), sulfamethoxazole (98%), sulfamethoxypyridazine (98%), sulfaquinoxaline (99%), sulfathiazole (98%), and sulfisoxazole (98%) were obtained from Sigma (St. Louis, MO, USA). The different standard solutions, prepared daily by dilution of a stock solution of each sulphonamide at a concentration of 0.6 g L^{-1}, were prepared by dissolving the pure substances in methanol. These solutions were stored in the dark and refrigerated at 4 °C. Sulfamethoxydiazine (SMTD), at a concentration of 5 μg L^{-1}, was used as the internal standard (IS). Magnetite embedded in phenyl silica adsorbents was synthesized by the sol–gel method using magnetite, phenyltrimethoxysilane (97%, PTMS) and tetramethylorthosilicate (98%, TMOS) from Sigma. Emulsion polymerisation of the monomers was performed using Triton X-100 and cetyltrimethyl ammonium bromide (CTAB) from Sigma.

2.2. Apparatus

The structures of the products obtained were determined by X-ray diffraction (XRD) using a Philips type powder diffractometer fitted with a Philips PW 1710 control unit, Vertical Philips PW 1820/00 goniometer and FR 590 Enraf Nonius generator (Philips, Almelo, Netherlands). The instrument was equipped with a graphite diffracted beam monochromator and copper radiation source (λ(Kα1) = 1.5406 Å), operating at 40 kV and 30 mA. The X-ray powder diffraction pattern was collected by measuring the scintillation response to Cu Kα radiation versus the 2θ value over a 2θ range of 5–70, with a step size of 0.02° and a counting time of 2 s per step.

The solid products were characterised using a Fourier transform infrared (FTIR) spectrophotometer (PerkinElmer, model IRDM). Dry samples were prepared in the form of KBr tablets (1:100, w/w).The morphological analysis of the magnetic adsorbents was performed using a JEOL JEM-1011 Transmission Electron Microscope (TEM) and JEOL JSM-6360LV scanning electron microscope (SEM; JEOL (Europe) B.V. Belgium).

The HPLC–DAD analyses were carried out on a Waters 2695 HPLC system, which is a liquid chromatography connected to a Waters 996 diode array detector (DAD) (Milford, MA, USA), and the dates were collected using Waters Empower Pro software. The separation was performed using a Gemini 3 μ C18 110 Å column (50 × 4.60 mm, 3 μm) supplied by Phenomenex (Macclesfield, UK). The samples were injected automatically (10.0 μL). Their separation was performed using two different mobile phases: an aqueous solution of 0.1% formic acid (Phase A) and a solution of 0.1% formic acid in acetonitrile (Phase B). The mobile phase gradient used was as follows: step 1: 0–4 min, 2% B, step 2: 4–5 min, 2–10% B, step 3: 5–27 min, 10–30% B, step 4: 27–30 min, 30–55% B, step 5: 30–30.5 min, 55% B, step 6: 30.5–31 min, 55–2% B, step 7: 31–35 min, 2% B. The total flow rate was kept constant at 0.5 mL/min during the separation. The chromatogram was monitored at 260 nm, and UV spectra of individual peaks were recorded in the range of 200–400 nm.

2.3. Synthesis of the adsorbent

The synthesis of the adsorbents was carried out in two steps. First, magnetite was obtained through the partial oxidation and precipitation of Fe(II) in the presence of oxygen in basic media (Barrado, Prieto, Vega, & Fernández-Polanco, 1998). Fifty millilitres of 1.25 mM $FeSO_4{\cdot}7H_2O$ solution were stirred at 60 ± 5 °C, and the solution was adjusted to a pH of 10 ± 0.2 with 6 M NaOH solution. After 1 h, the magnetic precipitates were isolated and washed with deionized water. The magnetic adsorbents were obtained by