Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1140 (2007) 147–156
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Validation of a liquid chromatography–mass spectrometry multi-residue method for the simultaneous determination of sulfonamides, tetracyclines, and pyrimethamine in milk

Urairat Koesukwiwat, Siripastr Jayanta, Natchanun Leepipatpiboon *

Chromatography and Separation Research Group, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

Received 12 July 2006; received in revised form 9 November 2006; accepted 27 November 2006

Abstract

A multiresidue method suitable for confirmation and determination of six sulfonamides (SAs), three tetracyclines (TCs), and pyrimethamine (PYR) in cow milk was validated. Milk samples were extracted using copolymer Oasis HLB solid-phase extraction (SPE) and analyzed by liquid chromatography–electrospray mass spectrometry with positive ion mode. Estimated method detection limits (MDL) and method quantitation limits (MQL) ranged from 0.48 to 2.64 and 0.61 to 8.64 ng/mL, respectively. These values are far lower than the maximum residue limits (MRLs) established by several control authorities. Excellent linear dynamic range was observed from the method quantitation limits to 300 ng/mL with correlation coefficients better than 0.9900 for all compounds. The method was accurate with recoveries ranging from 72.01 to 97.39%. Good intra-precision and intermediate precision were obtained with RSD better than 11.08%. The method is fairly robust with sample pH being the only critical control point.

Keywords: Sulfonamide; Tetracycline; Pyrimethamine; Milk; Antibiotics; LC-ESI-MS

1. Introduction

At present, the occurrences of drug residues especially antibiotics in foods and foodstuffs originating from veterinary uses have become increasingly apparent. Sulfonamides (SAs) and tetracycline (TCs) are broad spectrum antibiotics frequently used in Thailand as veterinary medicines. They are commonly used for the prevention and treatment of dairy cattle for several infectious diseases, prophylactic, or as feed additives to promote growth in farm animals [1]. Because SAs exhibit antibacterial synergy with pyrimethamine (PYR), these medicines are frequently co-administered for amplified effects [2]. Structures of SAs, TCs, and PYR are illustrated in Fig. 1.

Milk is known as a nutritious wholesome food consumed globally. Because it is an inexpensive source of protein and calcium essential for promoting growth in children and general good health of the population, its consumption has been considerably promoted in Thailand. However, due to poor hygienic conditions of many local dairy farms, cows often get ulcers or sores on their udders. This bovine condition is known as *mastitis* and requires antibiotics to treat and control the condition. Prevalent use of antibiotics in dairy farming such as unnecessary administration for disease control and not following label directions are major sources of veterinary drug residues in milk. The presence of these drug residues, regardless of their minute amounts, can trigger potential adverse side effects in humans such as allergic reactions in hypersensitive individuals, other long-term health effects, or they can be potential carcinogenic. Prolonged exposure to residue antibiotics can result in an increase of drug-resistant bacteria [1]. Therefore, monitoring of antibiotic residues is very important in controlling the safety of milk for human consumption. For these reasons, several control authorities such as the European Union (EU), the U.S. Food and Drug Administration (FDA), and the Thailand Ministry of Public Health (MOPH) issue strict maximum residue limits (MRLs) that allow only trace amounts of antibiotic residues in milk for human consumption [3–5]. An implementation of the effective monitoring program requires specific, sensitive, and reliable

* Corresponding author. Tel.: +66 2 2187608; fax: +66 2 2541309.
E-mail address: natchanun.l@chula.ac.th (N. Leepipatpiboon).

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.11.099

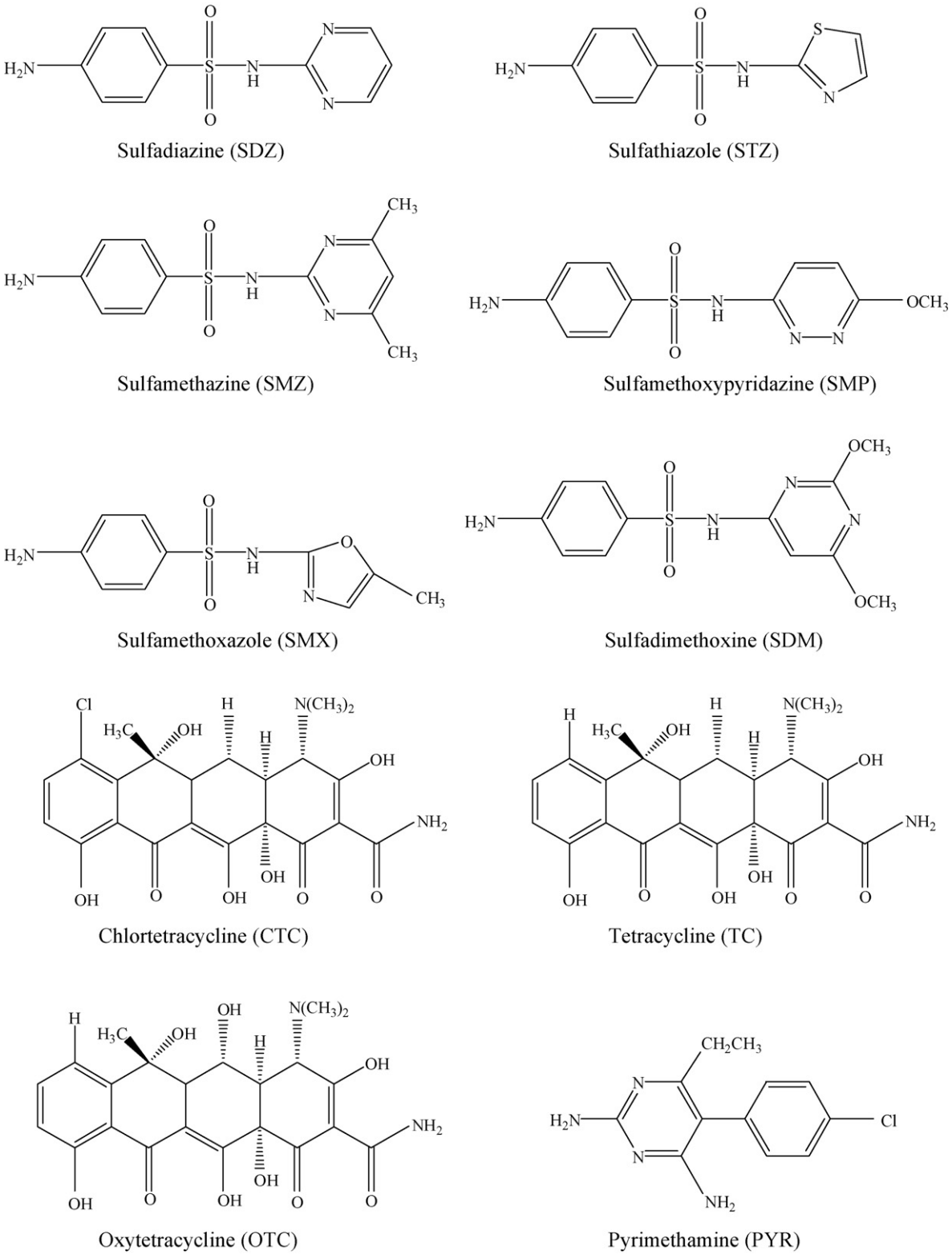

Fig. 1. Chemical structures of sulfonamides, tetracyclines, and pyrimethamine.

analytical methods that can detect all drug residues below these regulated levels.

It is possible to analyze SAs, TCs, and PYR in foods. Microbiological and immunological [6,7] assays are commonly used for rapid screening. However, these techniques are complicated, lack sensitivity and specificity, and thus are only suitable for semi-quantitative measurements. Liquid chromatography (LC) coupled with UV [8–11], DAD and FLD [12,13] emerged as attractive alternatives for the determination of antibiotics due to their higher selectivity, sensitivity, and precision. GC [14]