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Fast extraction of amphenicols residues from raw milk using novel fabric phase sorptive extraction followed by high-performance liquid chromatography-diode array detection

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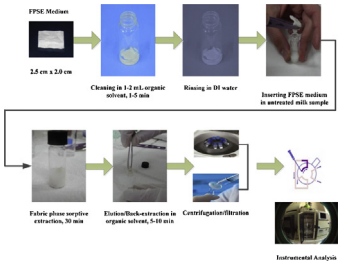
HIGHLIGHTS

- Fabric phase sorptive extraction-HPLC method developed for amphenicols in milk.
- FPSE is a simple, fast, sensitive and green sample preparation approach.
- FPSE eliminated protein precipitation step, reducing steps in sample preparation.
- Sol–gel PEG coated FPSE media provided high efficiency extraction of amphenicols.
- FPSE offers new capability to routinely monitor three amphenicols in milk samples.

GRAPHICAL ABSTRACT



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ABSTRACT

A simple, sensitive, reliable, and fast analytical method was developed for the simultaneous determination of amphenicols residues in raw milk by combining fabric phase sorptive extraction (FPSE) and high-performance liquid chromatography-diode array detection. FPSE, a new generation green sample preparation technique, efficiently incorporates the advanced and tunable material properties of sol–gel derived microextraction sorbents with the rich surface chemistry of a cellulose fabric substrate, resulting in a flexible, highly sensitive, and fast microextraction device capable of extracting target analytes directly from complicated sample matrices. Due to the strong chemical bonding between the sol–gel sorbent and substrate, the microextraction device demonstrates a very high chemical and solvent stability. Therefore, any organic solvent/solvent mixture can be used as the eluent/back-extraction solvent.

Herein, a highly polar polymer coated FPSE media was created using short-chain poly(ethylene glycol) (PEG) and the applicability of this novel microextraction device to extract highly polar amphenicol antibiotics from raw milk was investigated. Due to the intense affinity of amphenicols towards the strongly polar sol–gel PEG-coated FPSE device, absolute recovery of the selected antibiotics residues were found to be 44% for thiamphenicol, 66.4% for florfenicol, and 81.4% for chloramphenicol. The developed method was validated in terms of sensitivity, linearity, accuracy, precision, and selectivity according to

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European Decision 657/2002/EC. Decision limit (CCα) values were 52.49 μg kg^{-1} for thiamphenicol, 55.23 μg kg^{-1} for florfenicol, and 53.8 μg kg^{-1} for chloramphenicol, while the corresponding results for detection capability (CCβ) were 56.8 μg kg^{-1}, 58.99 μg kg^{-1}, and 55.9 μg kg^{-1}, respectively.

1. Introduction

Milk is one of the most universally consumed foods, necessary for human growth and well-being. As a sample matrix, milk is considered to be very complex, consisting of water, lactose, protein, fat, minerals, and vitamins [1]. The food quality of milk is frequently impaired due to the prevalent use of antibiotics in veterinary medicine. Amphenicols e.g., thiamphenicol (TAP), florfenicol (FFC), and chloramphenicol (CAP), whose chemical structures and relevant physicochemical properties are shown in Table 1, are synthetic antibiotics with a similar broad-spectrum of activity, but not usually effective against *Pseudomonas*. They are often administered to animals for disease prevention; however, they also show toxic effects in humans. Chloramphenicol is hematotoxic and may cause severe adverse effects, such as bone marrow aplasia, which leads to aplastic anemia [2]. The use of chloramphenicol in foodstuffs has been banned within the European Union, and the minimum required performance limit (MRPL), set by European Decision181/2003/EC, is 0.3 μg kg^{-1} [3]. It is also prohibited to give florfenicol to milk producing animals, while the MRPL for thiamphenicol (TAP) is 50 μg kg^{-1} [4]. Therefore, expensive analytical instruments are often inevitable for the quantitation of amphenicols residues at such low concentrations. However, no matter how sophisticated the analytical instrument used, sample preparation is still a compulsory and determinative step in chemical analysis.

For the isolation of amphenicols in food, particularly in milk, various sample pre-treatment techniques have been proposed, including polymer monolith microextraction (PMME) [5], molecularly imprinted polymer microspheres (MIPMs) [6,7], liquid–liquid extraction [8], matrix solid-phase dispersion (MSPD) [9], and SPE [10–14].

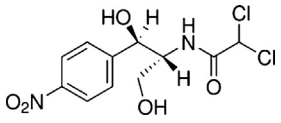
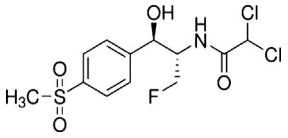
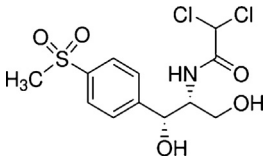
Thiamphenicol was determined in milk after using the following extraction procedure: extraction using Extrelut-3 with ethyl acetate, evaporation of the extract, reconstitution in 40% acetonitrile, and finally quantitation by HPLC-UV [15]. Amphenicols were extracted with ethyl acetate and hexane, followed by cleaned up with a silica cartridge. Recovery was found to range from 68.0 to 90.0% and detection limits were at the 300 ppb level [16]. A similar procedure was followed using a one step liquid-extraction with ethyl acetate and a subsequent C_{18}-dispersive, solid phase extraction for further clean-up [17].

Molecularly imprinted polymer monolith microextraction (MIPMME) was also applied for thiamphenicol in milk. The MIP monolith synthesized in a micropipette tip could be connected with syringes of different sizes to simply perform solid-phase extraction processes without any other treatment [18].

Deproteinization of the milk was applied, followed by sample enrichment, and cleanup by continuous solid-phase extraction [19]. A modified matrix solid-phase dispersion (MSPD) procedure was applied for the extraction and clean-up procedure of antibiotics, using a mixture of Strata (Phenomenex) and QuEChERS as a sorbent, to avoid the deproteinization step prior to SPE [9].

A careful examination of the physicochemical properties of amphenicols revealed that all of the amphenicols are inherently polar and strongly hydrophilic. It is a well-known fact that isolating highly polar analytes from an aqueous solution is still considered to be a daunting task [20,21]. Although the current trend in analytical sample preparation clearly favors miniaturized solvent-less/solvent-minimized equilibrium driven microextraction techniques, surprisingly, the majority of analytical methods developed thus far for amphenicols antibiotics are based on solvent intensive, exhaustive extraction techniques. Such techniques often consume a high volume of toxic and hazardous organic solvents and

Table 1
Chemical structures and relevant physiochemical properties of selected amphenicols.

Amphenicol	Abbreviation	Molecular weight	Molecular structure	log K_{ow}
Chloramphenicol	CAP	322.01		1.14
Florfenicol	FF	358.21		0.8
Thiamphenicol	TAP	355.00		−0.3