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Short communication

Method optimization and validation for the determination of eight sulfonamides in chicken muscle and eggs by modified QuEChERS and liquid chromatography with fluorescence detection

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ABSTRACT

A simple, effective and reliable method for the determination of eight sulfonamide antibiotics (sulfadiazine, sulfapiridine, sulfamerazine, sulfamethazine, sulfachloropiridazine, sulfamethoxazole, sulfadoxine, sulfadimethoxin) in chicken muscle and eggs by liquid chromatography and fluorescence detection has been developed and validated. Sulfonamides do not present native fluorescence, however their direct determination was achieved by on-line post-column photochemical derivatization by UV irradiation. Sample treatment was based on QuEChERS with several modifications depending on the matrix. Egg extracts were cleaned-up using PSA for the dispersive solid phase extraction step. On the other hand, a new clean-up sorbent, Supel^{TM} QuE Z-Sep^{+}, has been successfully applied in chicken muscle extract and has proved to be effective for interference removal from this matrix. Under optimum conditions, recoveries from 65.9 to 88.1%, relative standard deviations lower than 10% (except for sulfachloropiridazine), and limits of quantification (LOQs) from 14 to 85 $\mu g\,kg^{-1}$ were achieved. Thus, the method complies with current European requirements.

1. Introduction

Sulfonamides (SAs) are a group of synthetic antimicrobials that are frequently employed for clinical and veterinary purposes, in order to prevent the growth of bacteria and treat the infections from certain microorganisms and protozoa. After tetracyclines, SAs are the most commonly used veterinary antibiotics within the EU because of their main characteristics, such as broad antibacterial spectrum, high efficacy, and low cost [1,2]. SAs can be either administered directly to livestock or added to feeds to prevent and treat gastrointestinal and respiratory diseases. The presence of SA residues in the food chain is of increasing concern because their adverse effect in human health, such as allergic reactions in hypersensitive individuals, their potential carcinogenic character and the possible development of antibiotic resistance, so efficient analytical methods are required [1,3,5]. To safeguard human health, the European Union (EU) has established a maximum residue limit (MRL) of 100 $\mu g\,kg^{-1}$ for the total amount of SAs in foods from animal origin such as muscle [4]. On the other hand, the use of SAs in animal producing eggs for human consumption is not allowed, and therefore the "zero tolerance" principle is applied for these matrices. Therefore simple and reliable analytical methodologies are needed to ensure consumer food safety.

Several analytical approaches have been reported for the determination of SA residues in different foodstuffs at the concentration level required by EU regulations, although liquid chromatography (LC) has been the technique most widely used [3,5]. Recently, the coupling of LC with mass spectrometry (MS) has played an important role in the control of SAs and other contaminant residues because of the high sensitivity, selectivity and the unambiguous identification capability offered by this technique [3,6–9]. However, LC–MS presents some drawbacks such as the high instrumental cost and the occurrence of abundant matrix effects, which may compromise the quantitative and selectivity performance of the methods [3]. Other cost-effective detection systems have also proved to be suitable for the determination of SAs in food samples after a LC separation, as for instance fluorescence (FLD), which is by nature highly selective and much more sensitive than UV-absorbance [3].

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As SAs do not present native fluorescence a derivatization step is required in order to reach enough sensitivity for the determination of these analytes. Chemical derivatization with fluorescamine has been the method typically employed for fluorescence labeling [10–13]. However, photochemical derivatization of SAs with UV irradiation, which induces a great fluorescence enhancement for heterocyclic SAs, has been also reported [14,15]. This approach has been applied on-line in flow injection analysis [16,17] and recently in our laboratory as a post-column derivatization mode in LC [18].

Last trends in sample treatments for the determination of SAs in edible animal tissues include, among other, liquid–liquid and solid-phase extraction (SPE), dispersive liquid–liquid microextraction and the so-called Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) sample preparation procedure [3], which has been widely applied as pre-treatment for the determination of SAs in different matrices [6,8,9,19,20].

The aim of this work was the optimization and validation of a simple and reliable analytical method for the determination of eight SAs (sulfadiazine, sulfapiridine, sulfamerazine, sulfamethazine, sulfachloropiridazine, sulfamethoxazole, sulfadoxine, sulfadimethoxin) in food matrices of animal origin such as chicken muscle and eggs. For this purpose, an on-line photochemical reactor was connected after the chromatographic column, which allowed the use of affordable LC-FLD instrumentation. With this approach a time consuming extra step to derivatize SAs during sample preparation is avoided. Efficient and very simple sample treatments based on QuEChERS methodology have been optimized for each type of matrix. The analytical procedure described in this

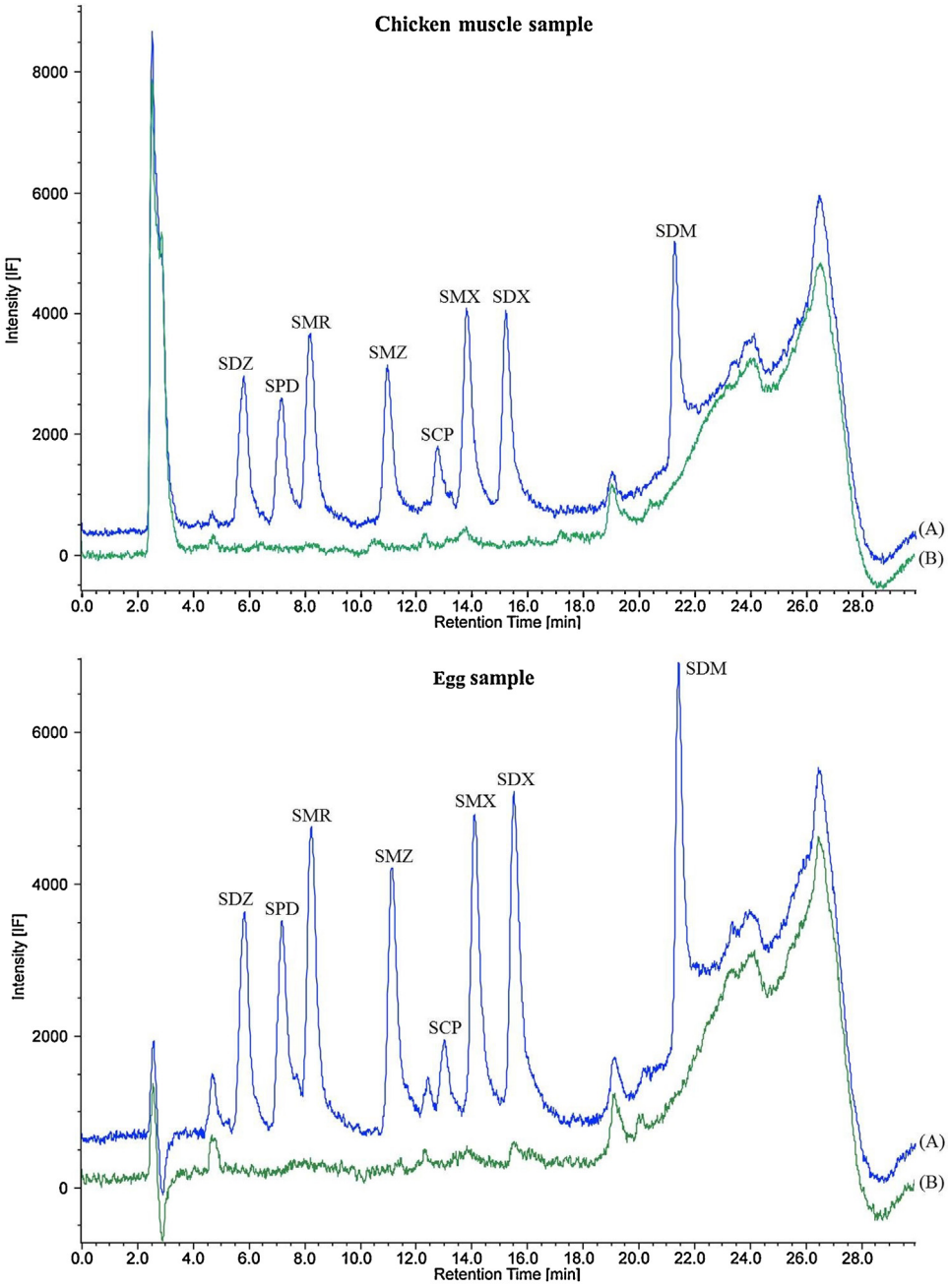

Fig 1. Chromatograms corresponding to a chicken muscle and an egg sample analysed by the proposed method (A: spiked with 250 μg kg^{-1} of each SA; B: blank). SDZ: sulfadiazine; SPD: sulfapiridine; SMR: sulfamerazine; SMZ: sulfamethazine; SCP: sulfachloropiridazine; SMX: sulfamethoxazole; SDX: sulfadoxine; SDM: sulfadimethoxin.