Food Chemistry 272 (2019) 148–156

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Development and validation of a method for simultaneous determination of trace levels of five macrocyclic lactones in cheese by HPLC-fluorescence after solid–liquid extraction with low temperature partitioning

Ana Maria de Souza Santos Cheibub[a,b], Eduardo Silva Bahiense de Lyra[a], Annibal Duarte Pereira Netto[a,b,*]

[a] Fundamental and Applied Analytical Chemistry Laboratory (LAQAFA), Department of Analytical Chemistry, Chemistry Institute, Fluminense Federal University (UFF), Outeiro de São João Batista, s/n, 24020-141, Valonguinho, Centro, Niterói, RJ, Brazil

[b] Graduate Program in Chemistry, Institute of Chemistry, Fluminense Federal University, Outeiro de São João Batista, s/n, 24020-141, Valonguinho, Centro, Niterói, RJ, Brazil

ARTICLE INFO

Keywords:
Acrocyclic lactones
Avermectin
Milbemycin
Veterinary drug residues
HPLC-fluorescence
Cheese analysis
Solid-liquid extraction with low temperature partitioning (SLE-LTP)

ABSTRACT

A highly sensitive analytical method was developed and validated, following international guidelines, for the determination of the residues of five macrocyclic lactones (MLs) (abamectin, doramectin, eprinomectin, ivermectin and moxidectin) in cheese. The extracts were concentrated by rotary-evaporation and derivatized; no clean-up was necessary. Despite matrix complexity, no significant matrix-effect was verified, and standards were prepared in solvents. Linear working ranges varied from 0.25 to 5.0 $\mu g\,L^{-1}$. Excellent limits of quantification (0.58–0.87 $\mu g\,kg^{-1}$), mean recoveries (91–103%), and repeatability and intermediate precision (< 5.8%) were obtained. Twenty-two samples of bovine and non-bovine cheeses were analyzed. Twenty-one samples showed residues of at least one ML (between 0.59 and 15.3 $\mu g\,kg^{-1}$), but moxidectin was never detected; a sample of mozzarella was free of MLs. To the best of our knowledge, this is the first method describing the simultaneous evaluation of these MLs in cheese using HPLC and fluorescence detection.

1. Introduction

Abamectin (ABA), doramectin (DOR), eprinomectin (EPR), ivermectin (IVM) and moxidectin (MOX) are macrocyclic lactones (MLs), which are widely used in veterinary medicine to control of a broad spectrum of parasitic infections in food producing animals. ABA, DOR, EPR and IVM are part of the avermectin family (AVM), which is produced by fermentation of the soil bacterium *Streptomyces avermitilis*. The structure of AVM consists of a 16-member macrocyclic ring containing a spiroketal group, a benzofuran ring and disaccharide functionality (Danaher, Howells, Crooks, Cerkvenik-Flajs, & O'Keeffe, 2006). MOX is a member of milbemycin family, which is produced by the bacterium *Streptomyces cyanogriseus* or *S. hygroscopicus*, (Prichard, Ménez, & Lespine, 2012) and presents a structure like that of AVM, but it lacks the disaccharide group (Danaher et al., 2006). These MLs share similar pharmacological activities and chemical characteristics, so they are typically grouped in monitoring studies.

The success of MLs in the pharmaceutical field is associated with their good efficacy against endo- and ectoparasites, even at low doses (Giannetti et al., 2011). Moreover, owing to their lipophilic proprieties, MLs have long-term persistence in the animal body. Several pharmacokinetic studies are available for these compounds, reporting a large volume of distribution, long residence time and elimination in milk during lactation (Cerkvenik et al., 2002, 2004; Bassissi, Alvinerie, & Lespine, 2004; Wen et al., 2010; Campbell, Pairis-Garcia, Campler, Moraes, & Mccutcheon, 2017). When present in milk, ML contamination may persist in dairy products. Studies have shown that MLs are stable under common processing through heating used in the dairy industry (Imperiale et al., 2009; Cerkvenik, Doganoc, Skubic, Beek, & Keukens, 2001), and in cooking conditions (as microwave heating, frying or boiling) (Rose, Farrington, & Shearer 1998). They also resist acid fermentation (Cerkvenik et al., 2004) and freezing at −20 °C (Cerkvenik et al., 2001). Residues of veterinary drugs in the food chain pose a potential risk to public health and are perceived as undesirable by consumers (Imperiale et al., 2006). Therefore, several organizations, such the European Union (European Commission Regulation, 2010),

* Corresponding author at: Department of Analytical Chemistry, Chemistry Institute, Fluminense Federal University (UFF), Outeiro de São João Batista, s/n, 24020-141, Valonguinho, Centro, Niterói, RJ, Brazil.
E-mail addresses: annibal@vm.uff.br, annibalnetto@id.uff.br (A.D. Pereira Netto).

https://doi.org/10.1016/j.foodchem.2018.08.027
Received 16 March 2018; Received in revised form 18 July 2018; Accepted 7 August 2018

Codex Alimentarius (2017) and Brazilian National Sanitary Surveillance Agency (ANVISA, 2012) have established maximum residue limits (MRLs) or tolerance levels for MLs in milk and other edible tissues (muscle, liver, kidney and fat) designated for human consumption.

A positive correlation of ML concentrations with fat and solid contents of dairy products was previously demonstrated (Cerkvenik et al., 2004, Imperiale, Busetti, Suárez, & Lanusse, 2004; Macedo et al. (2015a). The results obtained in determination of MLs in butter indicated that the high lipid content of the samples concentrated these compounds (Macedo et al., 2015a). The number of contaminated samples and the residual concentrations of MLs found in butter (Macedo et al., 2015b) were proportionately much higher than those found in milk in Brazil, which typically are below the legal limits (Furlani et al., 2014; Rübensam, Barreto, Hoff, Kist, & Pizzolato, 2011; Lobato, Rath, & Reyes, 2006). In sheep, buffalo and goat cheese production, the highest concentrations of MLs were measured in curds, and were up to 4 times higher than in the milk samples employed for their production (Anastasio et al., 2002; Cerkvenik et al., 2004; Imperiale et al., 2004).

The determination of ML residues in products of animal origin requires methods with high selectivity and sensitivity because the concentrations of interest are typically very low. The analytical approaches used to detect residues of MLs in food and biological matrices were reviewed by Danaher and colleagues (Danaher et al., 2006; Danaher, Radeck, Kolar, Keegan, & Cerkvenik-Flajs, 2012). Methods based on high-performance liquid chromatography (HPLC) have been widely used to determine these compounds in milk, urine, feces, plasma (Danaher et al., 2006) and animal tissues such as liver, muscle and fat (Wang et al., 2011; Inoue, Yoshimi, Hino, & Oka, 2009). Fluorescence detection is possibly the most commonly method used for ML determination and has been preferred for routine activities, due to the high comparative costs of HPLC coupled to mass spectrometry (HPLC-MS) (Trapero, Yepes, Romero, & Carrasquila, 2016).

There are few studies about ML determination in cheese. The works of Anastasio et al. (2002), Imperiale et al. (2004), Imperiale et al. (2006) and Cerkvenik et al. (2004) were based in experiments with animals that were previously treated with MLs. They aimed to describe the pattern of ML excretion in milk and other tissues after drug administration and to establish the correlation between the ML concentrations present in milk and cheese produced from the milk samples. The methods were based on HPLC-Fluo and studied the presence of one or two MLs per sample. The authors employed the same solid phase extraction (SPE) method as for milk, cheese and other biological matrices. However, no method optimization or metrological studies were presented in these articles for the cheeses studied. Only one method based on ultra-high-performance liquid chromatography with mass spectrometry detection (UPLC-MS/MS) was developed and validated for the determination of ML residues in cheeses (Pérez, Romero-Gonzalez, Vidal, & Frenich, 2013). The multiresidue method included 17 veterinary drugs, among them three MLs (IVM, DOR and emamectin) (Pérez et al., 2013). However, this required high implementation cost, due to MS/MS detection and sample treatment based on QuEChERS, which presents satisfactory recoveries for polar substances in complex matrices but can be expensive because a specific sorbent and internal standards are necessary (Lehotay et al., 2010).

To date, there are no methods available for the simultaneous determination of a set of MLs in bovine cheeses using HPLC-Fluo, with fast and low-cost sample preparation. Such a method would allow systematic monitoring of cheeses available for consumption. The adequate treatment of the cheese samples to obtain appropriate analytical figures of merit was one of the former challenges of this study because cheeses are complex matrices compared to milk, since cheeses usually contain larger quantities of proteins and fat than milk, while butter is formed mostly by solvent soluble lipids (Macedo et al., 2015a). Thus, the more complex matrices of cheeses can affect all analytical procedures (from sample processing to analyte determination) (Pérez et al., 2013). To work around this issue, a method of solid–liquid extraction and low temperature partitioning (SLE-LPT) was employed in this study. SLE–LTP is similar to liquid–liquid extraction with low temperature partitioning (LLE–LTP) (Rübensam, Barreto, Hoff, & Pizzolato, 2013), which has been introduced for food processing as a simple and cost-effective alternative extraction method, in which no clean-up step is necessary, and has been successfully employed for milk analysis (Rego et al.,2015; Rübensam et al., 2011).

This study presents the development, validation and application of a highly sensitive analytical method based on SLE-LTP and HPLC-Fluo for the simultaneous determination of residues of five MLs (EPR, MOX, DOR, ABA and IVM) in cheeses of different origins and characteristics. As far as we know, this is the first published study aiming at method validation and application for simultaneous determination of these MLs in cheese.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile, methanol and isopropanol, all HPLC grade, were purchased from J.T. Baker (Center Valley, PA, USA). High purity water (resistivity of 18.2 MΩ cm) was obtained using a Sartorius Arium Comfort II system (Göettingen, Germany). The derivatization reagents (purity > 99 wt%) trimethylamine (TEA), 1-methyimidazole (MI), trifluoroacetic anhydride (TFAA) and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Anhydrous magnesium sulfate (P.A. grade), supplied by VETEC (Rio de Janeiro, RJ, Brazil), was pulverized using a mortar and pestle and heated overnight at 250 °C before use.

Abamectin (98.7 wt%), doramectin (95.4 wt%), eprinomectin (94.1 wt%), Ivermectin (94.0 wt%) and moxidectin (97.1 wt%) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and employed as standards without further purification.

2.2. Preparation of solutions and standards

Standard stock solutions of the individual MLs (100 mg L^{-1}) were prepared by dissolving 1.0 mg of each compound in 10 mL of acetonitrile. They were stored at −20 °C in amber glass flasks and discarded one month after preparation. A multi-compound solution was prepared by combining suitable aliquots of each individual standard stock solution in acetonitrile to obtain final concentration of 5.0 mg L^{-1} for each ML. This solution was kept at −20 °C and renewed every week. Working standard solutions (50 μg L^{-1} and 25 μg L^{-1}) were prepared daily by appropriate dilutions of the multi-compound solution with acetonitrile.

2.3. Description of samples

Nineteen samples of different types of bovine cheese - Ball, namely blue, Brie, Camembert, *Coalho* (typical Brazilian cheese), Emmental, Gouda, cured *Minas* (typical Brazilian cheese), Mozzarella, Parmesan, *Prato* (typical Brazilian cheese) and Provolone – one sample of buffalo cheese (Mozzarella), one sample of ovine cheese (Pecorino) and one sample of caprine cheese were purchased from local supermarkets in the cities of Niterói and Rio de Janeiro (RJ, Brazil). All samples were stored at −20 °C and analyzed following the procedures described below (Sections 2.4, 2.6 and 2.7).

2.4. Sample preparation

Frozen (−20 °C) samples of cheese were ground using a domestic blender (Philips Walita RI1364, 400 W). Aliquots of 1.0 g of each studied cheese were weighted at room temperature in polypropylene Falcon tubes (50 mL) and submitted to solid–liquid extraction with low