Food Chemistry 150 (2014) 328–334

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Ultrasound-assisted dispersive extraction for the high pressure liquid chromatographic determination of tetracyclines residues in milk with diode array detection

Eftichia Karageorgou, Marina Armeni, Ioulia Moschou, Victoria Samanidou *

Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki 541 24, Greece

ARTICLE INFO

Article history:
Received 28 June 2013
Received in revised form 7 October 2013
Accepted 1 November 2013
Available online 13 November 2013

Keywords:
Tetracyclines
Milk
Ultrasound
Dispersion
Chromatography

ABSTRACT

Ultrasound assisted matrix solid phase dispersive extraction was applied for the selective isolation and clean-up of tetracyclines (oxytetracycline, tetracycline, epi-chlorotetracycline, chlorotetracycline and doxycycline) from milk. Target analytes were determined by an accurate and sensitive chromatographic analytical method, which was validated to meet the European Legislation criteria. The separation was performed on a LiChroCART-LiChrospher® 100 RP-18 (5 μm, 250 × 4 mm) analytical column, operated at ambient temperature, followed by diode array detection.

Validation included investigation of linearity, selectivity, stability, limits of detection and quantitation, decision limit, detection capability, trueness, precision and ruggedness according to the Youden's approach. Limits of quantitation of examined tetracyclines were from 14.5 to 56.6 μg/kg significantly lower than respective Maximum Residue Limits, whereas recoveries ranged from 82.0% to 108%. The applicability of the method was evaluated using milk samples purchased from local market. Accuracy of the method was additionally proved by analysis of bovine milk certified reference material (BCR®-492).

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Antimicrobials of tetracyclines's group (TCs) were first discovered in 1945. They are broad-spectrum antibiotic agents extensively used to control bacterial infections in humans and animals. They exhibit activity against infections caused by both Gram-positive and Gram-negative bacteria, chlamydia, mycoplasmas, rickettsiae and protozoan parasites. TCs are given to animals destined for human consumption to promote growth. However the presence of residues in milk or edible animal tissues may potentially cause allergic reactions or may lead to toxic and dangerous effects on human health (Samanidou, Nikolaidou, & Papadoyannis, 2007a).

In order to protect human health, the European Union (EU) has enacted maximum residue limits (MRLs) for the presence of TCs in foodstuff of animal origin. The use of veterinary drugs in the EU is regulated by Commission Regulation (EU) No. 37/2010 (2009), which describes the procedure for the establishment of MRLs for veterinary medicinal products in food, whereas Decision 2002/657/EC (European Commission Decision 2002/657/EC, 2002), defines the performance criteria and the interpretation of results for analytical methods in the official control of residues in products of animal origin (Samanidou, Nikolaidou, & Papadoyannis, 2007b).

Analysis of tetracyclines in milk is performed mainly by HPLC with various detection techniques (Cinquina, Longo, Anastasi, Gianneti, & Cozzani, 2003; Frenich, Anguilera-Luiz, Vidal, & Romero-Gonzalez, 2010; Fritz & Zuo, 2007; Furusawa, 2003; Kaale, Chambuso, & Kitwala, 2008; Kishida, 2011; Robert et al., 2013; Samanidou et al., 2007a; Shariati, Yamini, & Esrafili, 2009; Tsai et al., 2010; Young & Tran, 2011; Zhao, Zhang, & Gan, 2004), although Flow Injection Analysis and immunochemical techniques have been also proposed (Gao, Zhao, Wang, & Wang, 2013; Rodriguez, Espinosa, Aguilar-Arteaga, Ibarra, & Miranda, 2010).

Sample pre-treatment techniques used for the isolation of tetracyclines from milk include Solid Phase Extraction (SPE) (Fritz & Zuo, 2007; Furusawa, 2003) with prior deproteinization using TCA, EDTA, and EDTA-McIlvaine buffer solution (Cinquina et al., 2003; Samanidou et al., 2007b; Young & Tran, 2011), liquid–liquid extraction (Kaale et al., 2008; Zhao et al., 2004) and magnesium hydroxide co-precipitation method (Tsai et al., 2010)

Recently, for the isolation of tetracyclines from milk a carrier mediated hollow fiber liquid phase microextraction was used (Shariati et al., 2009), and for the same purpose a magnetic solid phase extraction (MSPE) was performed, which involves the extraction and clean-up by silica based magnetic support dispersion on non-pretreated milk samples, followed by the magnetic isolation and desorption of the analytes by acidified methanol (Rodriguez et al., 2010). Also isolation of the target compounds

* Corresponding author. Tel.: +30 2310997698; fax: +30 2310997719.
E-mail address: samanidu@chem.auth.gr (V. Samanidou).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.11.008

was achieved by using centrifugal ultrafiltration device with Ultrafree-MC/PL [low binding regenerated cellulose membrane, nominal molecular weight limit (NMWL) = 5000, capacity <0.5 mL, without prior sample preparation (Kishida). These pre-treatment techniques demand either specific equipment not available in every analytical laboratory, or high consumption of solvents and long time of sample preparation. Nowadays, for the isolation of target compounds of complex matrices like milk, the trend is to use simple, fast, of low cost and almost solvent-free sample preparation techniques. Such a technique is Matrix Solid Phase Dispersion (MSPD). By a thorough search in the literature, MSPD was used only once for the isolation of tetracyclines residues from egg with no success according to the authors (Frenich et al., 2010). Recently matrix solid-phase dispersion extraction was applied for the determination of tetracycline residues in milk by capillary electrophoresis (Mu, Liu, Xu, Tian, & Luan, 2012). The uniqueness of MSPD as sample preparation technique is that it's especially suitable for the extraction of solid, semi-solid and/or highly viscous food and biological matrices, consisted in obtaining isolation of target analytes by dispersing sample matrix onto a solid support. In this way any difficulty encountered by employing the classical SPE approach is eliminated. MSPD is a flexible technique regarding selectivity because various SPE sorbents can be used, allowing also combination of them. Sonication can assist the process of analytes extraction and sample clean-up as it provides an efficient contact between the solid and the extractant, which typically result in higher recovery rates of the target analytes (Karageorgou & Samanidou, 2010).

The authors have previously introduced the combined use of ultrasound power in matrix solid phase dispersion (MSPD) with new sorbent materials like QuEChERS for the preparation of samples with satisfactory results for the multi-residue determination of other antimicrobials namely cephalosporins, penicillins, amphenicols and quinolones in milk (Karageorgou & Samanidou, 2010; Karageorgou, Myridakis, Stephanou, & Samanidou, 2013; Karageorgou & Samanidou, 2011; Karageorgou, Samanidou, & Papadoyannis, 2012).

In this study ultrasound assisted matrix solid phase dispersion is proposed for the determination of five tetracyclines by HPLC-DAD. Target analytes are oxytetracycline (OTC), tetracycline (TC), epi-chlorotetracycline (epi-CTC), chlorotetracycline (CTC) and doxycycline (DC).

To the best of our knowledge, this is the first attempt that ultrasound assisted matrix solid phase dispersion is applied successfully on the isolation of tetracyclines residues from milk. Validation of the proposed method was performed according Commission Decision 2002/657/EC, determining linearity, selectivity, stability, decision limit, detection capability, trueness, precision and ruggedness according to the Youden's test approach. Limits of detection and quantitation were also calculated although not required by Commission Decision 2002/657/EC for the integrity of the study.

The bias of the method was further proved by the analysis of a certified reference material.

2. Materials and methods

2.1. Instrumentation

A quaternary low pressure gradient HPLC–DAD system was used for the chromatographic analysis, purchased from Shimadzu (Kyoto, Japan). The solvent lines were mixed in an FCV-10ALVP mixer. An LC-10ADVP pump equipped with a Shimadzu SCL-10ALVP System Controller, permitting fully automated operation, was used to deliver the mobile phase to the analytical column. Sample injection was performed via a Rheodyne 7725i injection valve (Rheodyne, Cotati California, USA) equipped with a 20 μL loop. Detection was achieved by an SPD-M10AVP photodiode array detector, supplied with data acquisition software Lab Solutions-LC solutions by Shimadzu. Degassing of the mobile phase was achieved by helium sparging in the solvent reservoirs by a DGU-10B degassing unit.

A glass vacuum filtration apparatus obtained from Alltech Associates was employed for the filtration of ammonium acetate, using Whatman cellulose nitrate 0.2 μm membrane filters (Whatman Laboratory Division, Maidstone, England). A Glasscol small vortexer (Terre Haute, IN, USA) and an ultrasonic bath Transonic460/H (35 kHz, 170 W, Elma, Germany) were employed for the pretreatment of milk samples. All evaporations were performed with a ReactiVap 9-port evaporator model 18780 by Pierce (Rockford, IL, USA). Q-Max RR syringe filters (0.22 μm nylon membrane) used were purchased from Frisenette ApS (Knebel, Denmark).

A LiChroCART-LiChrospher® 100 RP-18 (5 μm, 250 × 4 mm) analytical column by Merck (Darmstadt, Germany) was used for the chromatographic separation.

Four SPE products were investigated towards their efficiency for the isolation of tetracyclines from milk: Nexus Abselut (60 mg/3 mL) by Agilent Technologies Inc., (Santa Clara, CA, USA), Oasis-HLB (200 mg/6 mL) by Waters (Milford, Massachusetts, USA), Merck-Lichrolut RP-18 (200 mg/3 mL) by Merck and Bond Elut Plexa (60 mg/3 mL) by Agilent Technologies Inc., 2 mL dispersive SPE tubes QuEChERS containing 150 mg magnesium sulfate, 50 mg PSA (primary, secondary amines) and 50 mg C_{18}EC were purchased by Agilent Technologies Inc., and were used in MSPD mode.

Certified reference material (BCR®-492) used, (consisted of 10 mL of a dry residue of lyophilised skimmed milk) was obtained from a bovine animal treated with oxytetracycline. This material has been certified by BCR (Community Bureau of Reference, EU, Geel, Belgium).

2.2. Chemicals and reagents

Tetracycline (purity) >98% (TC), oxytetracycline hydrochloride >95% (OTC) and Chlorotetracycline hydrochloride >97% (CTC) were purchased from Fluka Chemie (Buchs, Belgium). Doxycycline hyclate >98% (DC) and caffeine (IS) were purchased from Sigma (Steinheim, Germany). epi-Chlorotetracycline hydrochloride >97% was purchased from Acros Organics (New Jersey, USA).

HPLC grade methanol and acetonitrile were obtained from Fisher Scientific (Steinheim, UK). Oxalic acid was supplied by Merck. High purity water, obtained by a Milli-Q purification system (Millipore, Bedford, MA, USA), was used throughout study.

2.3. Chromatography

Target analytes were separated by gradient elution using A: 0.01 M Oxalic acid and B: ACN. The initial volume ratio 85:15 (v/v) changed to 70:30 (v/v) within 12 min and finally remained stable for 5 min. Flow rate was set at 1.2 mL/min providing an inlet pressure of 160–180 bar.

Column performance was evaluated by calculation of number of theoretical plates N, tailing factor T_f, relative retention time RRT, retention factor k, resolution factor Rs, and the precision of the retention time and peak area.

Peak identification was performed by spectral information provided by the diode array detector. Monitoring and quantitation was performed at 355 nm for tetracyclines and at 275 nm for the IS.

Download English Version:

https://daneshyari.com/en/article/7599504

Download Persian Version:

https://daneshyari.com/article/7599504

Daneshyari.com