Talanta 148 (2016) 321–328

Contents lists available at ScienceDirect

Talanta

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

Development and application of a dispersive liquid–liquid microextraction method for the determination of tetracyclines in beef by liquid chromatography mass spectrometry

S.O.S. Mookantsa [a,b], S. Dube [a], M.M. Nindi [a,*]

[a] Department of Chemistry, College of Science, Engineering and Technology, University of South Africa, P.O. Box 392 UNISA, Pretoria, South Africa
[b] Residue Section, Botswana National Veterinary Laboratory, P/Bag 0035, Gaborone, Botswana

ARTICLE INFO

Article history:
Received 20 April 2015
Received in revised form
2 November 2015
Accepted 3 November 2015
Available online 4 November 2015

Keywords:
Dispersive liquid-liquid microextraction
Sample clean-up
Sample preparation
Mass spectrometry
Dispersive solid phase microextraction
Tetracycline

ABSTRACT

A rapid, cost effective and environmentally friendly extraction method, based on dispersive liquid–liquid microextraction (DLLME) was developed for the determination of six tetracyclines in meat destined for human consumption. Meat extracts were analysed for tetracyclines using liquid chromatography tandem mass spectrometry (LC–MS/MS), a sensitive and selective analytical technique. Various factors influencing the pre-concentration of tetracyclines such as sample pH, type and volume of both disperser solvent and extraction solvent were optimized. Validation parameters such as calibration function, limit of detection (LOD), limit of quantification (LOQ), detection capability (CCα), decision limit (CCβ), accuracy and precision were established according to Commission Decision 2002/657/EC. Linearity in the range of 25–200 μg kg^{-1}was obtained with regression coefficients ranging from 0.9991 to 0.9998. Recoveries of spiked blank muscle samples at three levels (50, 100 and 150 μg kg^{-1}) ranged from 80% to 105% and reproducibility was between 2% and 7%. LODs and LOQs ranged from 2.2 to 3.6 μg kg^{-1} and from 7.4 to 11.5 μg kg^{-1} respectively while CCα ranged from 105 to 111 μg kg^{-1} and CCβ ranged from 107 to 122 μg kg^{-1}. The proposed method compared well with the existing accepted dispersive solid phase extraction method and was successfully applied to the pre-concentration and determination of tetracyclines in meat samples. Eleven of the thirty bovine muscle samples obtained from local abattoirs and butcheries were found to contain residues of two tetracycline antibiotics (chlortetracycline and oxytetracycline), with oxytetracycline being the most detected. Concentration levels of the tetracycline residues detected in bovine muscle samples were lower (12.4 and 68.9 μg kg^{-1}) than the stipulated European Union maximum residue level (MRL) of 100 μg kg^{-1}, hence the meat was fit for human consumption. From this work it can be concluded that the DLLME is indeed a greener sample preparation method and could be used as an alternative to dispersive solid phase microextraction (dSPE).

1. Introduction

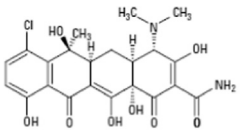
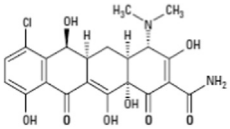
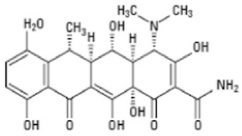
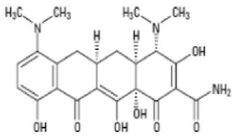
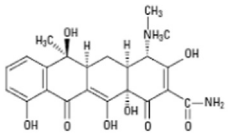
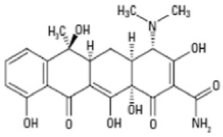
Tetracylines (TCs) produced by *Streptomyces* spp. are a large family of antibiotics used in human and veterinary medicine that have a broad-spectrum activity against most Gram-positive and Gram-negative bacteria including some anaerobes. TCs are actively transported into the cells of susceptible bacteria where they bind to the 30S ribosomal sub-particle. In this way, protein synthesis is inhibited, which explains their bacteriostatic effect. Oxytetracycline (OTC), tetracycline (TC), chlortetracycline (CTC), doxycycline (DXC), demeclocycline (DMC) and minocycline (MNC) are (Table 1) commonly used in food producing animals, due to their broad-spectrum activity, cost effectiveness, availability and ease of administration. When tetracycline drugs are administered by laymen, as in most cases, correct dosages and withdrawal periods are unlikely to be observed. This raises concerns that animal tissue intended for human consumption might be contaminated with residues of TCs [1–3]. Human health problems resulting from intake of subchronic exposure levels of TCs include gastrointestinal disturbances, poor foetal development, hypersensitivity and other toxic effects. Moreover, resistant strains of staphylococci, coliforms, bacilli, heumococci, haemolytic streptococci, *Haemophilus influenczae* and *Clostridium welchii* have been reported [1–3], thus causing a huge challenge to human health.

In order to safeguard human health, organisations such as the World Health Organisation (WHO), the Food Agriculture Organisation (FAO) and European Union (EU) have set standards for

* Corresponding author.
E-mail addresses: sandyseedu2007@yahoo.com (S.O.S. Mookantsa), dubes@unisa.ac.za (S. Dube), nindimm@unisa.ac.za (M.M. Nindi).

http://dx.doi.org/10.1016/j.talanta.2015.11.006
0039-9140/

Table 1
Structures and physico-chemical properties of tetracyclines compounds studied.

Tetracycline	Structure	MW	pKa
Chlortetracycline		478.1143	3.3, 7.4, 9.3
Demeclocycline		464.0986	3.3, 7.2, 9.3
Doxycycline		444.533	3.1, 7.7, 9.3
Minocycline		457.1849	3.3, 7.2, 9.3
Oxytetracycline		460.1482	3.3, 7.3, 9.1
Tetracycline		444.1533	3.3, 7.7, 9.7

acceptable daily intake and maximum residue limits (MRLs) of these veterinary drugs in foods. The acceptable MRLs are set at 100 μg kg^{-1} for muscle, 300 μg kg^{-1} for liver and 600 μg kg^{-1} for kidney for all food-producing animals [4–7].

Sample preparation is the most critical step in analysis because it determines the accuracy and precision of the analytical method. The most common methods used for sample clean-up in the determination of tetracycline residues are liquid–liquid extraction (LLE) and solid phase extraction (SPE) [8,9]. These traditional sample extraction and clean-up methods are expensive, complicated, laborious, time consuming and require large amounts of organic solvents. Some of these organic solvents are well documented to be toxic, carcinogenic and are costly to dispose. Recent research on sample preparation is focused towards the development of efficient, economical, miniaturised and environmentally friendly methods to overcome the limitations of traditional sample preparations. These methods include solid phase microextraction (SPME) [10,11], dispersive solid phase microextraction (dSPE) [12], liquid phase microextraction (LPME) [10], single-drop liquid-phase microextraction (SD-LPME) [13], hollow fibre-based liquid-phase microextraction (HF-LPME) [14], QuECHERS (Quick, Easy, Cheap, Effective, Rugged and Safe) [15] and dispersive liquid–liquid microextraction (DLLME) [16].

Dispersive liquid–liquid microextraction which was first reported by Assadi et al. in 2006 has emerged as an attractive alternative to the traditional extraction and clean-up method with its advantages of simplicity of operation, rapidity, low cost, high recovery and high enrichment factors [16–18]. This extraction technique has been applied successfully and widely for the extraction of target analytes including tetracyclines from aqueous samples which are less complicated [16,18]. Unfortunately, extracting analytes using DLLME from solid biological samples is more complex. Solid biological sample matrices such as muscles present a challenge in that there is need to first extract the analyte from a complex matrix prior to applying DLLME. Recently, there have been some modifications of this technique to include extraction and clean-up of various solid sample matrices in the analysis of veterinary drugs. For example, DLLME method was successfully applied in swine muscle and chicken liver for the determination of quinolones by high performance liquid chromatography [19,20]. The extraction efficiencies reported ranged from 50% to 90% while LODs and LOQs ranged from 5 to 19 μg kg^{-1} and 23 to 62 μg kg^{-1} respectively [20].

In this work, a rapid, cheap and simple, green extraction method, DLLME, was developed and validated according to Commission Decision 2002/657/EC implementing council Directive 96/23/EC, for the analysis of tetracyclines residues in bovine muscle samples using LCMS/MS [24]. The new method was compared with the South African National Accreditation System accredited dSPE method for accuracy and precision. To the knowledge of the authors this is the first time that such work on DLLME of tetracyclines in biological samples is being reported.

2. Experimental

2.1. Reagents and chemicals

Acetonitrile, methanol and formic acid were of LC–MS grade from Fluka (Steinheim, Germany) while acetone, chloroform, dichloromethane, trichloromethane and tetrachloroethylene were of HPLC grade from Merck (Darmstadt, Germany). Sodium hydroxide pellets, sodium chloride, trisodium citrate dihydrate and magnesium sulphate anhydrous, were all of analytical grade from Merck (Darmstadt, Germany). Chlortetracycline hydrochloride (97.8%), oxytetracycline hydrochloride (95%), tetracycline hydrochloride (95%), doxycycline hyclate (97%), demeclocycline hydrochloride (98%), minocycline hydrochloride and methacycline hydrochloride (95%) analytical standards were from Sigma-Aldrich (Steinheim, Germany). Ultra high purity water of 18.2 MΩ cm^{-1} resistivity was obtained from the Milli-Q purification system from Millipore (Bedford, MA, United States). Bond Elute C18 sorbent was from Agilent Technologies (Santa Clara, USA) and 0.45 μm PVDF membrane filters were from Pall Corporation (New York, USA).

2.2. Instrumentation

An Applied Biosystems 4000 Qtrap mass spectrometer was from Applied Biosystems/ABSciex (Pty) LTD (Darmstadt, Germany) and was used for all mass spectral measurements. The mass spectrometer was equipped with electrospray (ESI) interface operating in a positive mode. ESI parameters were optimized for each tetracycline by direct infusion of individual standard solution into the mass spectrometer (Table 2). The mass spectrometer parameters were; ion-source temperature (500 °C), ion-source gas 1 (50 psi), ion-source gas 2 (40 psi), curtain gas (25 psi) and collision gas (medium). The mass spectrometer was operated in the selective reaction monitoring (SRM) mode to confirm the identity of tetracyclines. This was achieved by selecting specific precursor-to-product ion for each tetracycline and quantifying using the most