Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Journal of Pharmaceutical and Biomedical Analysis 47 (2008) 487–493
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Capillary electrophoretic method for the simultaneous determination of tetracycline residues in fish samples

P. Kowalski *

Medical University of Gdańsk, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Hallera 107, PL-80-416 Gdańsk, Poland

Received 30 July 2007; received in revised form 17 January 2008; accepted 21 January 2008
Available online 2 February 2008

Abstract

A capillary electrophoresis (CE) method with UV detection was developed for simultaneous identification and quantitation of three tetracyclines: tetracycline (TC), oxytetracycline (OTC) and doxycycline (DC) in fish muscle samples. It was developed by analyzing a series of fish tissue samples containing TCs in different concentrations. The CE method was validated in terms of linearity, sensitivity, selectivity, decision limit (CCα), detection capability (CCβ), precision and accuracy for investigated TCs. The limits of detection (LODs) estimated in the range 1.3–1.8 ng/g, while the limits of quantification (LOQs) for all analyzed TCs were ranged from 4.3 to 5.9 ng/g. The overall recoveries for TC, OTC and DC were 84.0, 80.6 and 89.2%, respectively, with repeatabilities below 6.2% R.S.D. In this report the use of extended light path (ELP) silica capillary and aqueous buffer solutions are combined with low wavelength UV detection (ca. 200 nm) to obtain the necessary sensitivity for simultaneous TCs determination in biological samples.
© 2008 Elsevier B.V. All rights reserved.

Keywords: Tetracyclines; Fish muscle; Validation study; Extended light path capillary

1. Introduction

A survey on the use of chemotherapeutics in European Union (EU) identified tetracyclines (TCs) as one of the most used antimicrobial agents in fish farming. TCs are commonly applied to food-producing animals as veterinary medicines because of the broad spectrum activity against pathogenic microorganisms [1], relatively low degree of toxicity and low-cost. Their improper administration in veterinary medicine can leave residues in edible animal products and promote occurrence of antibiotic resistant bacteria. Therefore, methodology for the screening of antimicrobial agents that are used in fish farms need to be developed. The EU has set levels of maximum residue limits (MRL) for all substances belonging to the TC group in foods of animal origin (100 μg/kg for fish muscle).

Documented literature indicates that the identification and quantitation of TC residues in biological samples can present some problems. First, the isolation of these compounds from matrix proteins is notoriously difficult due to its ability to form chelates with metal ions as well to dehydrogenation, photodegradation or partial epimerization at the 4-position [2–5]. Secondly, they can be difficult to be extracted from biological matrix into organic solvents due to binding to protein. To overcome these problems, most extraction techniques from tissues use a strong acid or acidic deproteinizing agents. Both to improve the extraction efficiency and to avoid the inclusion of a competing chelating agent, the isolation of TCs was performed in the presence of sodium ethylenediamine tetraacetate (Na_2EDTA), citric or oxalic acids [5].

A number of methods for quantification of TC antibiotics in different matrices can be found in the literature. Due to low volatility of TCs, they are not suitable also for routine gas chromatographic (GC) analysis. Some of liquid chromatographic (LC) methods [6–11] are sensitive enough for quantification of TCs at very low levels, despite the difficulties associated with peak tailing and low efficiency, which are caused by the interaction with the residual silanol groups in the stationary phase. Simultaneous quantification of TCs in fish food products have been reported only in a few of the articles [7,10,12,13].

Because of the structural similarities of analytes, their ionic nature and acid–base properties complete resolution by chromatographic methods is not usually achieved. However, their

* Tel.: +48 58 3493136; fax: +48 58 3493130.
E-mail address: piotrpl@wp.pl.

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.01.036

water solubility and multiple ionization sites make them more amenable for electrophoretic separation. For determination of TCs a varied electrophoretic technique, including micellar electrokinetic chromatography (MEKC) [14,15], capillary zone electrophoresis (CZE) [16,17] and electrochromatography (CEC) [18] has been used. Despite all documented electrophoretic methods allow TCs determination in environmental samples [19] or in pharmaceutical preparations [20], there has been no electrophoretic method for simultaneous separation of the most used TCs in tissue samples.

In the present report the use of ELP silica capillary and aqueous buffer solution are combined with low wavelength UV detection (ca. 200 nm) to obtain the necessary sensitivity for simultaneous TCs determination in fish samples. This approach has been applied to the antibiotic residue analysis for the first time. The aim of this work was to developed simple and low-cost method, which does not involve complicated clean-up procedures and allowed TC residues determination in fish tissue samples at nanogram level. An experiment was also undertaken to obtain real fish samples containing TC antibiotics and to test the applicability of the electrophoretic method.

2. Experimental

2.1. Reagents and samples

All reagents used for the preparation of samples and running buffer were of analytical grade (methanol, acetonitrile, disodium ethylenediamine tetraacetate (Na_2EDTA), citric acid, boric acid, disodium hydrogenphosphate, monosodium phosphate, disodium tetraborate) were supplied by Merck (Darmstadt, Germany). Buffer solutions for separation were prepared using triple distilled water and were compound with boric acid (pH 5.6, 20 mM), monosodium phosphate (pH 5.7, 10 mM) and concentrated phosphoric acid (adjusted to pH 2.7). McIlvaine buffer solution (pH 4.0) was prepared by confounding 0.1 M citric acid (61.45%) with 0.2 M disodium hydrogenphosphate (38.55%). Tetracyclines (TC, OTC, DC, as their hydrochlorides) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ephedrine hydrochloride (used as internal standard, I.S.) was supplied by Farm-Impex (Gliwice, Poland).

Separate standard stock solutions of each TC compounds were prepared at a concentration of 1 mg/ml by dissolving the pure substances in 2 mM disodium tetraborate solution. These solutions were protected from light and stored under refrigeration (4 °C) until use to avoid possible decomposition and were stable for up to 7 days. Further solutions (working solutions) in the concentration range 1–100 μg/ml were prepared daily by dilution of the standard stock solution in triple distilled water. Calibration standards were prepared at concentrations of 0.005, 0.01, 0.02, 0.05, 0.1 μg/ml and finally 0.2 μg/ml of the mixed standard solution of each of the drugs.

2.1.1. Experimental fish

Healthy crucian carp (*Carassius auratus*) (mean body weight ± S.D.) 225 ± 38 g were obtained from the local fish farm. The fish were stocked into four outdoor experimental tanks: one group as control and three treatment groups for residue studies of different kind of TC compounds. In each group, the fish were kept in tanks with fresh water and the temperature was maintained at 15.0 ± 0.5 °C. The fish were supplied to acclimatize for 7 days prior to the start of experiment, were fed a commercial non-drugs feed and were starved for 1 day before experiment. Next, the three treatment group of animals were fed medicated feed with the therapeutic dose of 100 mg/kg body weight per day, for five consecutive days. Each of three fish group was fed with one kind of TC only. A few fish, used as control group, were given feed without the drug. Five fish were removed from each tank at days 14 and 21 after the cessation of the medication period. The skin and bones were removed, and the muscles were minced, saved and stored frozen at −20 °C until assay. No fish died in the TCs-administered group during the experiment period. Each sample was prepared twice and each of these was injected twice.

2.2. Instrumental parameters and conditions

The experiments were carried out on a capillary electrophoresis (CE) system P/ACE 2100 (Beckman Instrumental, Fullerton, CA) equipped with a UV detector connected to data collection system and autosampler. Samples were introduced from the anodic end of extended light path (ELP) capillary (57 cm length and 75 μm ID) with a bubble cell of 150 μm fused-silica capillary by 5 s hydrodynamic injection with pressure 3.45 kPa. The separations were performed using “normal” polarity (the cathode was located on the detector side). The capillary was thermostated in 22 °C and constant voltage of 20 kV was applied. At the beginning of each day, the capillary was conditioned with 0.1 M sodium hydroxide for 5 min, then rinsed with deionized water for 5 min and finally with running buffer (5 min). The capillary was flushed between runs with 0.1 M hydrochloric acid (0.2 min), next 0.1 M sodium hydroxide (1 min) and finally with triple distilled water (1 min). In each measurement, the capillary was rinsed with the running buffer for 1 min prior to injection. Likewise, before analysis and between each run, the capillary was voltage pre-conditioned in order to give high precision and to produce a stable baseline.

2.3. Procedures and sample preparation

For each analysis, an accurately weighed 3 g amount of the thawed muscle sample was homogenized, using a Polytron TM (Germany), at high speed for 3 min with 3 ml of mixture 0.1 M Na_2EDTA, McIlvaine buffer (pH 4.0) (1:2, v/v). The homogenized probe was washed with acetonitrile (3 ml), next shaken (5 min) and vortexed (2 min). The homogenate was centrifuged at 8000 rpm min^{-1} for 5 min, decanted to a clean polypropylene tube, and evaporated under reduced pressure at 45 °C to dryness. Dried extracts were reconstituted in 200 μl mixture of methanol and running buffer (1:1, v/v), centrifuged again with the same conditions as before and stored to analysis at −20 °C.

For routine analysis, negative control samples were fortified with the three analyzed drugs at each of the six levels of 0.005, 0.01, 0.02, 0.05, 0.1 and 0.2 μg/g. After fortification, samples

were held for 10 min prior to extraction procedure, and was prepared as the same above steps.

3. Results and discussion

Although TCs have been studied extensively with numerous detection modes, this work is a first example of validation of procedures for simultaneously quantifying this class of antibiotics at nanogram levels by CE. In this study was used a fused-silica capillary with bubble detection cell, which is highly sensitive detection cell with longer path-length at detector than conventional capillary ones. Moreover, the sensitivity has been improved by employing low wavelength UV detection (in the range 190–210 nm), where many solutes have enhanced UV activity. To evaluate the best wavelength useful for simultaneously TCs detection the absorbance spectra using diode array detection between 190 and 300 nm were performed. As shown in the inset of Fig. 1b the analytes have got the maximum absorbance in the region at 200 and 280 nm. Determination of TCs is generally carried out with UV absorbance detection at around 270–280 nm. Unfortunately, this range detection usually suffers from limited sensitivity and biological sample interference in some cases. Likewise, the high transparency of the silica capillary wall allows the use of low wavelengths which successfully may compensate the poor sensitivity in CE. The HLPC application method is limited by the short wavelength detection, which is known to make most of the commonly used mobile phase organic modifiers to absorb the UV light very well. Aqueous electrolytes that present with a low UV absorbance coefficient allow the detection at this range to be routinely used in the CE.

Preliminarily, the effects of injection volume, capillary temperature and applied voltage were investigated in order to ascertain any influence on the TCs quantifications. The sample extracts were analyzed by CE using hydrodynamic injection times of 2, 5, 7 and 10 s. It was observed that 5 s injections (corresponding to approximately 20 nl injection volume of sample) that led to separation of all TCs were most efficient. Likewise, it was verified that the temperature (in range 15–30 °C) and applied voltage (in range 15–30 kV) had important influence on the electrophoretic migration of analytes and separation efficiencies. Thus, temperature of 22 °C and 20 kV applied voltage were chosen as the best compromise between running time, efficiency of separation and selectivity. Moreover, the effect of capillary length (range examined: 47–67 cm) on TCs separation was also studied. It was found that a fused-silica capillary with effective length of 57 cm gives a satisfactory separation in a reasonable analysis time (6 min).

3.1. Isolation from biological matrix

The isolation of the analyzed antibiotics from biological samples was investigated based on the structures of molecules and their solubility properties as well was optimized to permit elimination of time-consuming purification steps. Acetonitrile was found as the best solvent for deproteination and extraction of analytes from tissue samples. In the previous studies effect of pH sample on the extraction efficiency was examined using several acidic (citric, oxalic and phosphoric) buffer solutions over the range 2–6. The optimum extraction efficiency was obtained at the pH range 2.5–2.7. Unfortunately, TC compounds are relatively stable in mildly acidic conditions but not in bases and under strong acid conditions (under pH 3.0 it can form epimers) [12]. Therefore, to maintain the feasible analysis of TCs, the fish tissue samples were adjusted to pH 4.0. However, to obtain the optimal conditions and the best recoveries of all analyzed compounds the combination of acetonitrile with EDTA–McIlvaine's buffer (pH 4.0) was chosen as extraction solvent. The solution of Na_2EDTA was added to prevent the well-known interference of TCs with multivalent metal ions. Likewise, Na_2EDTA has a greater affinity for the cations than TCs, causing improved recoveries of analytes when it is incorporated into the extraction solution [4]. A substantial increase in recovery of TCs was observed on lowering the pH by adding McIlvaine buffer solu-

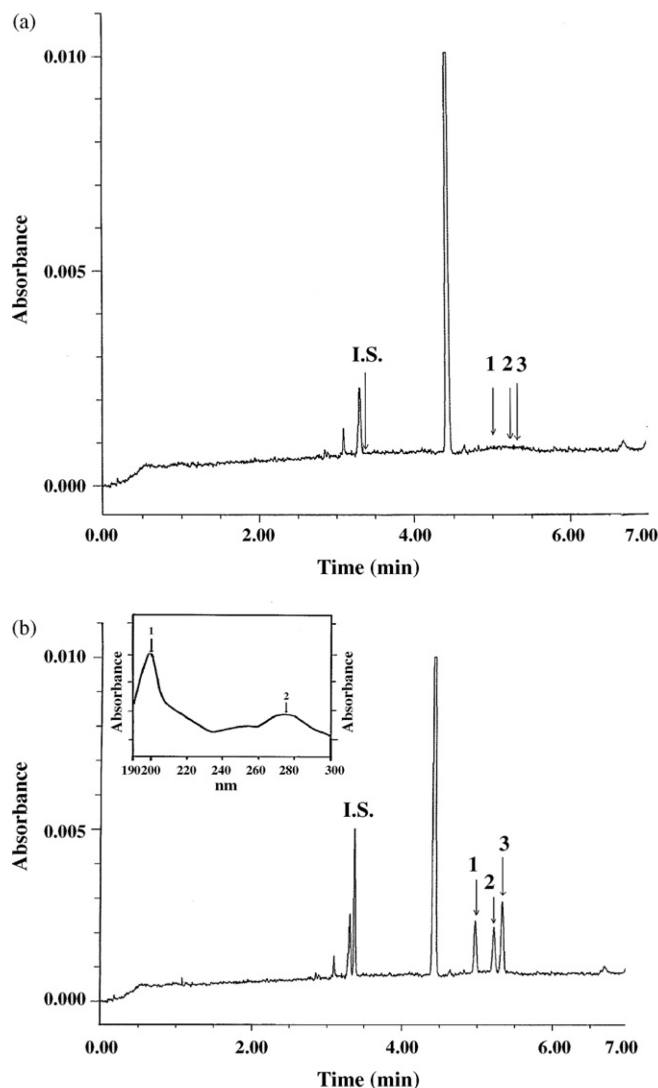

Fig. 1. (a) Typical electropherograms of drug-free (control) fish muscle sample. (b) Electropherogram of fish muscle sample spiked with ephedrine hydrochloride (I.S.) and tetracycline (1), oxytetracycline (2), doxycycline (3) at 50 ng/g of each of the antibiotic; UV spectrum for the mixture of analytes in running buffer.

tion (pH 4.0). Moreover, the combination of EDTA–McIlvaine buffer offered the best recovery lower limit of detection (LOD), so it was selected for the proposed experiments.

3.2. Buffer composition

To obtain the optimized separation of all analytes, the combined effects of buffer pH value, buffer concentration and the nature of TCs were taken into consideration. TC compounds can exist in the hydrated solid state as well as in aqueous organic solutions as the uncharged free base form and an equilibrium mixture of a zwitterionic form. Their chemical behaviour is depended upon the conditions of the medium (type and buffer concentration, pH value, ionic strength and temperature) [4]. Due to the fact, that TCs have three pK_a values (3.3/7.7/9.3), in strongly acidic pH, their molecule exists in its fully protonated form as a singly charged cation. Likewise, alkaline medium TCs are known to be easily oxidised, thus an acidic electrophoretic buffer should be used in order to avoid broad peaks resulting from oxidation of analytes. Although several of the electrolytes (citric acid and formic acid) provided a good separation of analytes, the best resolutions between peaks of determinated compounds and biological matrix were achieved by using the phosphate–borate solutions. Moreover, the best peak shapes were obtained over the pH range 2.4–3.0 and the electropherograms showed a generally flatter baseline than those obtained previously, facilitating both integration and interpretation. Optimization of buffer pH to affect CE separation was based on pK_a value of analytes. Likewise, TCs were hardly resolved with the buffer at pH value of 3.5, partially resolved at 3.0 and was well separated at 2.7. Next, the optimization of the separation was studied by manipulation of buffer concentration ranging from 10 to 50 mM both phosphate and borate electrolytes (keeping the pH values at 2.7). Complete separations (resolution <1.5) of all analytes was achieved by using a buffer composed with boric acid (20 mM), monosodium phosphate (10 mM) and concentrated ortho-phosphoric acid (adjusted to pH 2.7). TCs appear well resolved from the signal at the beginning and centre of the electropherograms, which correspond to the proteins and other tissue matrix components. At these operating conditions, the migration time of the internal standard was 3.38 min, while the migration times of TC, OTC, DC were 4.98, 5.23 and 5.34 min, respectively.

3.3. Validation of analytical methods

The CE method for TCs determination in fish samples was validated in compliance with the analytical performance parameters required for analytical method validation, including selectivity, accuracy, precision, linearity, LOD, limit of quantification (LOQ), recovery and stability. The assay has been carried out on the basis of the replicate analysis of samples containing known amounts of each drug at $0.5 \times$ MRL, $1 \times$ MRL and $2 \times$ MRL values for fish muscle, according to EU guidelines and corresponding to quality control (QC) levels. Tissue samples of antibiotic-free fish samples were fortified at drugs concentrations of 0.05, 0.1 and 0.2 μg/g. Replicate samples of each fortified tissue control were analyzed to determine the percent recovery for each of the drug. The unfortified tissues were analyzed as negative control to confirm that neither drug was present in the blank muscle tissue and that no interferences were observed in electrophoretic view. The linearity between concentration and peak signal response was evaluated using extract solutions of concentration in the range of 0.005–0.2 μg/g.

The TC LODs and LOQs were determined based on the standard deviation (σ) and slope (S) evaluated from the calibration curve of each of the analytes. LOD = $3\sigma/S$, LOQ = $10\sigma/S$. The value of σ was calculated based on the experimental calibration curves ($n = 6$, each level) being the standard deviation of (b) intercepts of the regression line. Precision was determined by the analysis of six replicates of calibration control samples at each concentration level of calibration curves in the same day (within-day) and on ten consecutive days (between-day variability), while accuracy was determined in the same day only (within-day). Within-day and between-day variabilities were evaluated using back calculated concentrations. Precision as degree of repeatability and intermediate precision of an analytical method was expressed as the percent relative standard deviation (R.S.D.) values for statistically significant number of samples. Assay accuracy was assessed by calculating the estimated concentrations as a percent of the nominal concentrations. The results for the decision limit (CCα) and the detection capability (CCβ) were calculated and the absolute recoveries of analytes and their internal standard were determined by direct comparison of peak signals from extracted versus non-extracted fish tissue samples. As part of the validation, the freeze stability of TCs was evaluated by comparing the stability of samples containing analyzed substance that had been frozen, stored in the dark and thawed three times, with fish samples that were thawed only once.

The CCα—limit, at which it can be decided that a sample is truly violate with an error probability of α and CCβ—the smallest content at which a method is able, with an error probability of β, to detect the analyte were calculated using calibration lines. The concentration at the (b) intercept plus 2.33 times the standard deviation (S.D.) of the within-laboratory repeatability of the intercept equals the CCα. The CCβ value was calculated at the CCα plus 1.64 times the corresponding S.D. of the within-laboratory reproducibility of the mean measured content.

3.3.1. Selectivity

Selectivity of CE method was confirmed on the basis of the analysis of different drug-free muscle fish tissue samples and extracts of muscle spiked with each of the TCs at the MRL (100 ng/g). Typical electropherograms obtained with a negative muscle extract and sample containing the internal standard with analytes are illustrated in Fig. 1a and b, respectively. No interferences were observed during the electrophoretic run of the sample in the area where all TCs or internal standard peaks appear.

3.3.2. Linearity, range and sensitivity

The linearity of the method was evaluated by a calibration curve ($n = 6$) in the range of 0.005–0.2 μg/ml for each of the TC

Table 1
Quantitative performance TCs test for CE

	Tetracycline	Oxytetracycline	Doxycycline
Sample linearity (μg/g)		0.005–0.2	
Slope	13.849 ± 0.085	13.526 ± 0.085	16.479 ± 0.075
Intercept	0.100 ± 0.008	0.104 ± 0.008	0.088 ± 0.007
Standard error	0.014	0.014	0.012
LOD (ng/g)	1.7	1.8	1.3
LOQ (ng/g)	5.8	5.9	4.3
CCα (ng/g)	119	123	104
CCβ (ng/g)	132	136	116

Summary of precision and validation data for tetracyclines in fish muscle obtained with CE calibrations.

at six concentration levels, with triplicate analyses. Details of the concentration ranges and calibration curves are shown in Table 1. At the detection wavelength 200 nm the LOD values were ranged from 1.3 to 1.8 ng/g, whereas the limits of quantitation for each drug were proved in range 4.3–5.9 ng/g, with precision 12.1, 11.7, 12.7% and accuracy 103, 95, 104% for TC, OTC and DC, respectively. This method was sufficiently sensitive, with a quantification limit comparable [19,21] or lower [17,20] than for the earlier published CE methods with spectrophotometric detection.

3.3.3. Precision and accuracy

The values of within-day and precision and accuracy for all TCs are summarized in Table 2. The results were determined analysing the samples spiked with each of the substance at QC levels. The obtained results confirmed the validity of the method required for biological samples. The assay was investigated with respect to repeatability and intermediate precision. The within-day accuracies (calculated as the deviation of the mean from nominal concentration) for analyzed substance were better than between-day values. Migration time precision for all analyzed compound was acceptable, when expressed as a ratio between each of the analyte and I.S. migration time, being below 1.1%.

Table 3
Percent recovery of TCs from fortified fish muscle

Nominal concentration (μg/g)	Mean recovery (%) (n = 6)	Overall R.S.D. (%)
Tetracycline		
0.05	79.7 ± 3.3	
0.1	87.2 ± 2.6	4.9
0.2	85.2 ± 2.0	
Oxytetracycline		
0.05	78.3 ± 3.6	
0.1	81.5 ± 6.2	5.5
0.2	81.8 ± 2.4	
Doxycycline		
0.05	87.9 ± 2.8	
0.1	88.9 ± 3.9	3.5
0.2	90.7 ± 2.4	

3.3.4. Recovery

The absolute recoveries of TCs in fish muscle samples were determined at QC levels with six replicates for each concentration. Overall recoveries for TC, OTC and DC were 84.0, 80.6 and 89.2% with overall R.S.D. below 6% in all cases (Table 3). The absolute recovery of internal standard was 86.8% with R.S.D. below 2%. These data confirmed that the extraction provided adequate sensitivity to process the samples.

3.3.5. Stability tests

The stabilities of TCs and their I.S. in biological samples are affected by their chemical properties, the storage conditions and the matrix effect. For each of analytes, the freeze–thaw stability was evaluated using fish samples at QC levels. These tests were performed by measuring three replicates at each concentration during 2 months and obtained data have been confirmed that the analyte is stable in frozen tissue samples, when stored at −20 °C and could be handled under normal laboratory conditions without significant loss. Stability tests of OTC in biological matrix were carried out also by Croubels et al. [22] and gave similar results.

Table 2
Assay variability obtained from within-day and between-day experiments for tetracyclines

Nominal concentration (μg/g)	Within-day			Between-day		
	Concentration found (n = 6) (μg/g)	Precision R.S.D. (%)	Accuracy (%)	Concentration found (n = 6) (μg/g)	Precision R.S.D. (%)	Accuracy (%)
Tetracycline						
0.05	0.0513	5.5	102.6	0.0530	6.2	105.9
0.1	0.1004	3.9	100.4	0.1027	4.2	102.7
0.2	0.1997	1.4	99.8	0.1986	1.9	99.3
Oxytetracycline						
0.05	0.0510	4.8	102.0	0.0515	6.2	103.0
0.1	0.1014	2.9	101.4	0.1030	3.3	103.0
0.2	0.1992	2.0	99.6	0.1987	2.3	99.3
Doxycycline						
0.05	0.0492	4.7	98.4	0.0502	4.8	100.4
0.1	0.1006	3.3	100.6	0.1037	3.4	103.7
0.2	0.2000	1.1	100.0	0.1979	2.2	98.9

Table 4
Averaged muscle tissue concentration of TC residues in crucian carps given TCs in medicated feed for 5 days

	Drug concentration (ng/g)			
	14 days after the cessation of medication period ($n = 5$)		21 days after the cessation of medication period ($n = 5$)	
	Averaged residue	S.D.	Averaged residue	S.D.
Tetracycline	105	6	27	4
Oxytetracycline	122	6	32	3
Doxycycline	165	5	49	4

3.3.6. Robustness/ruggedness

The robustness of CE method was assessed by the eventual effects of small changes in the separating parameters (length of capillary, buffer pH value and temperature conditions). The method proved to be robust with respect to small changes in running buffer composition as was derived during CE method development (several freshly prepared buffer solutions were used during method validation). The migration time ranges (min) of internal standard, TC, OTC and DC during 10 days were 3.34–3.43 (R.S.D. = 1.1%), 4.94–5.02 (R.S.D. = 0.9%), 5.18–5.27 (R.S.D. = 1.1%) and 5.31–5.38 (R.S.D. = 1.0%), respectively. Small variations in temperature (22.0 ± 1.0 °C), using different capillaries, obtained from the same supplier did not significantly affect the separation in terms of resolution and peak shapes. The effect of capillary length (47–67 cm) and applied voltage (20–25 kV) on separation was also investigated. A good efficiency of all the analyte peaks were obtained with variations of 5–10% of the optimum values of electrophoretic parameters. It was therefore expected that routine use of CE method on this type instrument should not give rise to problems.

3.4. Analysis of real and commercial samples

The effectiveness of electrophoretic method was checked by analyzing muscle from the crucian carp, which were medicated with dose of 100 mg/kg per day of each of TCs for 5 days at 15 °C. The averaged concentration of three TC compounds in fish muscle samples is presented in Table 4. 21 days after the finishing of the therapy, the detected concentrations of drugs were lower than MRL values for TCs. The presented results are in general agreement with those of the previous studies for other fish species, where fish were fed OTC for at least 5–10 days. Postdosing OTC concentration values of 45 ng/g (20 days, at 18 °C), 40 ng/g (20 days, at 19–25 °C) and 32 ng/g (19 days, temperature below 9 °C) have been reported for the muscle of sea bream [23], ayu [24] and coho salmon [25], respectively. On the contrary, Ding et al. [26] reported high concentration of difloxacin in crucian carp muscle of 7.3 μg/g (at 10 °C) and 0.2 μg/g (at 20 °C) in 15 days after administration of a single dose (20 mg/kg body weight). It was proved by Wang et al. [27] in different study that OTC in a black sea bream in 22 day after end of the therapy (at 20.4 °C) was not detectable. Notwithstanding, a major part of these differences could be attributed to the different experimental conditions, like water temperature and period of feeding.

To demonstrate the usefulness of presented electrophoretic method, an experiment was performed also in muscle tissues of commercial fish (carp, crucian carp, trout, cod) obtained from local market. No samples contained detectable concentrations of analyzed TCs in the fish tested.

4. Conclusion

This work is a first example of a suitable electrophoretic method for the one-step extraction, and simultaneously detection of three TCs in fish samples. The analytical protocol proposed the use of ELP capillary, combined with low UV wavelength detection (200 nm) to obtain the required sensitivity. Relative to other reported procedures for these compounds, this approach offers greatly improved detection limits as well improved resolution. It has been confirmed in real biological samples research, that electrophoretic method may also be successfully applied to either for screening or quantitative analysis of TC residues in food samples of animal origin. It is concluded that the CE method developed has advantages over LC methods in terms of reduced sample pre-treatment, very little organic solvent, high separation efficiencies, speed of analysis as well as cost of operation.

References

[1] M.F.M. Tavares, V.L. McGuffin, J. Chromatogr. A 686 (1994) 129–142.
[2] W.J. Blanchflower, R.J. McCraken, A.S. Haggan, D.G. Kennedy, J. Chromatogr. B 692 (1997) 351–360.
[3] C.R. Anderson, H.S. Rupp, W.-H. Wu, J. Chromatogr. A 1075 (2005) 23–32.
[4] S. Huq, M. Garriques, K.M.R. Kallury, J. Chromatogr. A 1135 (2006) 12–18.
[5] R.D. Caballero, J.R. Torres-Lapasío, M.C. García-Alvarez-Coque, G. Ramis-Ramos, Anal. Lett. 35 (2002) 687–705.
[6] M. Turaki, P. Rigas, P. Pergandas, C. Kastritsis, J. Chromatogr. B 663 (1995) 167–171.
[7] A.D. Cooper, G.W.F. Stubbings, M. Kelly, J.A. Tarbin, W.H.H. Farrington, G. Shearer, J. Chromatogr. A 812 (1998) 321–326.
[8] R. Coyne, Ø. Bergh, O.B. Samuelsen, J. Chromatogr. B 810 (2004) 325–328.
[9] B.F. Spisso, A.L. de Oliveira e Jesus, M.A.G. de Araújo Júnior, M.A. Monteiro, Anal. Chim. Acta 581 (2007) 108–117.
[10] H. Nakazawa, S. Ino, K. Kato, T. Watanabe, Y. Ito, H. Oka, J. Chromatogr. B 732 (1999) 55–64.
[11] W.C. Andersen, J.E. Roybal, S.A. Gonzales, S.B. Turnipseed, A.P. Pfenning, L.R. Kuck, Anal. Chim. Acta 529 (2005) 145–150.
[12] Y. Wen, Y. Wang, Y.-Q. Feng, Talanta 70 (2006) 153–159.
[13] M.J. Schneider, A.M. Darwish, D.W. Freeman, Anal. Chim. Acta 586 (2007) 269–274.

[14] S. Croubels, W. Baeyens, C. Dewaele, C. Van Peteghem, J. Chromatogr. A 673 (1994) 267–274.
[15] Y.-C. Chen, C.-E. Lin, J. Chromatogr. A 802 (1998) 95–105.
[16] Y.M. Li, A. Van Schepdael, E. Roets, J. Hoogmartens, J. Pharm. Biomed. Anal. 15 (1997) 1063–1069.
[17] A. Van Schepdael, I. Van den Bergh, E. Roets, J. Hoogmartens, J. Chromatogr. A 730 (1996) 305–311.
[18] J.J. Pesek, M.T. Matyska, J. Chromatogr. A 736 (1996) 313–320.
[19] L. Nozal, L. Arce, B.M. Simonet, A. Ríos, M. Varcárcel, Anal. Chim. Acta 517 (2004) 89–94.
[20] M.C.V. Mamani, J.A. Farfán, F.G.R. Reyes, S. Rath, Talanta 70 (2006) 236–243.
[21] C.L. Chen, X. Gu, J. AOAC Int. 78 (1995) 1369–1377.
[22] S. Croubels, S. De Baere, P. De Backer, Anal. Chim. Acta 483 (2003) 419–427.
[23] J. Malvisi, G. Della Rocca, P. Anfossi, G. Giorgetti, Agriculture 147 (1996) 159–168.
[24] K. Uno, Aquaculture 143 (1996) 33–42.
[25] J.R. Meinertz, M.P. Gaikowski, G.R. Stehly, W.H. Gingerich, J.A. Evered, Aquaculture 198 (2001) 29–39.
[26] F. Ding, J. Cao, L. Ma, Q. Pan, Z. Fang, X. Lu, Aquaculture 256 (2006) 121–128.
[27] Q. Wang, Q. Liu, J. Li, Aquaculture 237 (2004) 31–40.